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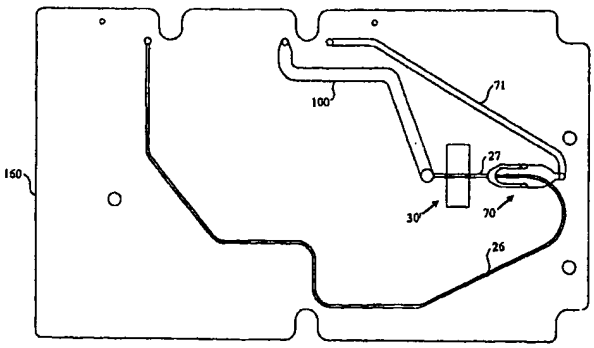
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(57) Abstract

The present invention provides an apparatus and method for storing a particle–containing liquid. The storage apparatus comprises a microfluidic convoluted flow channel (20) having a plurality of particle capture regions. The storage channel (20) is preferably an isotropic spatially periodic channel. Sedimented particles can be resuspended following storage. This invention further provides a microfluidic analysis cartridge (160) having a convoluted storage channel (20) therein. The sample analysis can use optical, electrical, pressure sensitive, or flow sensitive detection. A plurality of analysis channels (24A–24B) can be included in a single cartridge (160). The analysis channels (24A–24B) can be joined to reagent inlets for diluents, indicators or lysing agents. A mixing channel (80) can be positioned between the reagent inlet and the analysis region (30) to allow mixing and reaction of the reagent. The cartridge (160) can include additional valves (V1–V5) and pumps (P11–P15) for flow management. The analysis cartridge (160) can be a self–contained disposable cartridge having an integral waste storage container (100). This invention further provides a sheath flow assembly (70). The sheath flow assembly (70) includes a sample channel (26) and first and second sheath fluid channels positioned on either side of and converging with the sample channel. The assembly (70) also includes upper and lower sheath fluid chambers prositioned above and below and converging with the sample channel (26). The flow cartridges (160) of this invention can be formed by molding, machining or etching. In a preferred embodiment they are laminated.

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LIQUID ANALYSIS CARTRIDGE

FIELD OF THE INVENTION

This invention relates to microfluidic cartridges for analysis of liquid samples, and in particular to cartridges having a convoluted sample storage channel and to cartridges having a flow cytometric measuring region.

BACKGROUND OF THE INVENTION

With the advent of micro-machining technology, microfluidic devices have proliferated (for example, U.S. Patent No. 5,637,469 to Wilding et al., U.S. Patent No. 4,983,038 to Ohki et al., U.S. Patent No. 4,963,498 to Hillman et al., U.S. Patent No. 5,250,263 to Manz et al., U.S. Patent No. 5,376,252 to Ekstrom et al., E.P. Patent Publication 0381501B1, and Petersen, E. (1982) *Proc. of the IEEE*, vol. 70, No. 5, pp. 420-457). A practical limitation for particle-containing liquids such as blood is the sedimentation of particles within the device. Following loading the liquid in the device, appreciable particle sedimentation can occur within the time required to position the device in a measurement apparatus. For example, if the sample flow is slowed or stopped, blood cells can measurably settle out of plasma within 20 seconds. Without a sample management method and apparatus for sedimentation mitigation, quantitative analysis, especially using more than one analysis method sequentially, is impractical. Moreover, if samples are first collected and then transported to a measurement apparatus, as in a clinical setting or in field sampling, particle sedimentation can make accurate analysis impossible.

Microfluidic devices having sample storage reservoirs are known in the art (for example, E.P. Patent Publication 0381501B1). Because of particle sedimentation, these devices are useful only for samples without particles. Flow cytometric microfluidic devices are also known in the art (for example, U.S. Patent No. 4,983,038 to Ohki et al.). Flow cytometric measurements are specifically applicable to particle-containing liquids. However, without sedimentation mitigation the measurements can be performed only immediately following sample collection.

SUMMARY OF THE INVENTION

The present invention provides an apparatus and method for storing a particle-containing liquid. The storage apparatus comprises a fluidic convoluted flow channel having a plurality of particle capture regions therein. Particle capture regions are bends in the channel that provide local gravitational minima. When sample flow is arrested (i.e. stopped or slowed) during operation or storage, each of the particles sediments in the nearest particle capture region. Unlike a storage reservoir, the particles do not aggregate in a single clump. Because the particles are locally captured in a plurality of regions, it is possible to rapidly and effectively reconstitute the sample following sedimentation. The storage channel is preferably spatially periodic, where the term spatially periodic channel is used herein for a channel having a substantially constant number of particle capture regions per unit volume. Spatial periodicity facilitates sample reconstitution. The storage channel is more preferably an isotropic spatially periodic channel, where the term isotropic is used herein for a channel suitable for storing a particle-containing liquid regardless of channel orientation.

The particles can be resuspended by either a continuous or a reversing flow. For resuspension by continuous flow, the arrested sample flow is re-started and particles rejoin the sample fluid. The leading edge and trailing edge of the sample storage segments are discarded, but the middle segment is resuspended to a homogeneous mixture identical to the original sample. For the suspension by a reversing flow, a plurality of resuspension cycles are employed. Each resuspension cycle includes a dispense portion to sweep a volume of the stored sample, and an aspirate portion to sweep the volume in the opposite direction. Flow rates, swept volume and number of cycle are tailored to the sample fluid.

This invention further provides a fluidic analysis cartridge having a convoluted storage channel therein. The cartridge contains a sample inlet, a convoluted sample storage channel in fluidic connection with the inlet, an analysis channel, having an analysis region, in fluidic connection with the storage channel, and a valve interface positioned between the storage channel and the analysis region. The inlet includes an inlet shut-off interface to prevent leakage of the stored sample through the inlet. The cartridge further includes a resuspension pump interface to resuspend a sedimented sample by sweeping the sample from the storage channel in a continuous or reversing flow. The convoluted storage channel

enables accurate analysis of particle-containing samples. The sample analysis region provides for detection by any means known in the art, for example optical, electrical, pressure sensitive, or flow sensitive detection. For electrical detection, the cartridge can include an electrical interconnect. For optical detection, the cartridge can include a window positioned over the analysis region. The optical analysis can employ optical absorption, fluorescence, luminescence or scattering. Particularly useful are absorption and flow cytometric analyses.

A plurality of analysis channels can be included in a single cartridge. The analysis channels can be joined to reagent inlets to mix the sample with reagents such as diluents, indicators and lysing agents. The reagents can be fed into the cartridge using a pump, for example a syringe pump. The reagent can alternatively be stored in a reservoir in the cartridge. For microscale channels, having laminar flow, mixing of the reagent with the sample is predominantly diffusional mixing. A mixing channel can be positioned between the reagent inlet and the analysis region to allow mixing and reaction of the reagent with the sample. The cartridge can include additional valves and pumps for flow management. The analysis cartridge can be a self-contained disposable cartridge having an integral waste storage container to seal biological and chemical waste. The storage container can include a vent to release gases during fluid loading. The cartridge can have alignment markings thereon to facilitate positioning in an analysis instrument.

This invention further provides a disposable fluidic hematology cartridge and a method for using the cartridge. The hematology cartridge has both an absorption measuring channel and a flow cytometric measuring channel. The cartridge can include a convoluted storage channel. It can further include reagent inlets, mixing channels, a waste storage container, and valves and pumps. The flow cytometric measuring channel preferably has a means for forcing particles in the sample fluid into single file. This can be accomplished with a constricted flow passage. It is preferably accomplished using a sheath flow assembly.

This invention further provides a sheath flow assembly. The sheath flow assembly includes a sample channel and first and second sheath fluid channels positioned on either side of and converging with the sample channel. The assembly also includes upper and lower sheath fluid chambers positioned above and below and converging with the sample channel.

The sheath fluid channels provide hydrodynamic focusing in the widthwise direction, and the sheath fluid chambers provide hydrodynamic focusing in the depthwise direction. Because the assembly provides hydrodynamic focusing, geometric focusing is not required. It is not necessary for the sample channel to contract in either the widthwise or depthwise direction. Contracting channels can also be employed.

A sample analysis instrument for use with a fluidic analysis cartridge is further provided. The instrument includes a cartridge holder, a flow cytometric measuring apparatus positioned for optical coupling with a flow cytometric measuring region on the cartridge, and a second measuring apparatus positioned to be coupled with a second analysis region on the cartridge. The cartridge holder can include alignment markings to mate with cartridge alignment markings. It can also include pump mechanisms to couple with pump interfaces on the cartridge and valve mechanisms to couple with valve interfaces on the cartridge.

The convoluted storage channel provides one means for resuspending particles sedimented during sample storage. This invention also provides analysis cartridges having a storage reservoir and an alternative resuspension means. The resuspension means can be an ultrasonic vibrator acoustically coupled to the reservoir or a mechanical agitator either positioned within the reservoir or mechanically coupled to the reservoir.

The flow cartridges of this invention can be formed by any of the techniques known in the art, including molding, machining and etching. They can be made of materials such as metal, silicon, plastics and polymers. They can be formed from a single sheet, from two sheets, or, in a preferred embodiment, from a plurality of laminated sheets. This invention further provides a method of fabricating a laminated fluidic flow channel. In the method, flow elements are formed in rigid sheets and abutting surfaces of the sheets are bonded together. The term rigid sheet is used herein for a substantially inelastic sheet. A rigid material still exhibits flexibility when produced in thin sheets. The flow elements can include fluid channels within the plane of the sheet, vias (holes) to route the fluid to the next layer, analysis regions, pump interfaces and valve interfaces. The flow elements can be formed by methods including machining, such as die cutting or laser ablating, and molding. The sheets

can be bonded together by the use of an adhesive or by welding. They can alternatively be held together with mechanical compression.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1, comprising FIGS. 1A-B, is an analysis cartridge with a convoluted storage channel in (A) plan view and (B) cross section.

FIG. 2, comprising FIGS. 2A-B, shows convoluted storage channels with particle sedimentation for (A) an anisotropic storage channel and (B) an isotropic storage channel.

FIG. 3, comprising FIGS. 3A-D, are isotropic spatially periodic channels.

FIG. 4, comprising FIGS. 4A-B, is a pinch valve (A) unactuated and (B) actuated.

FIG. 5 is a syringe pump interface.

FIG. 6 is a plan view of a sheath flow assembly.

FIG. 7, comprising FIGS. 7A-G, shows the individual sheets which are laminated together to form the sheath flow assembly of FIG. 6.

FIG. 8 shows a reagent channel joining the sample channel.

FIG. 9 shows a convoluted mixing channel following the junction of a reagent channel with the sample channel.

FIG. 10, comprising FIGS. 10A-B, illustrates mixing of a particle-containing sample with a reagent in (A.) an anisotropic mixing channel and (B) an isotropic mixing channel.

FIG. 11 is a schematic drawing of an analysis cartridge having a convoluted storage channel and a plurality of mixing and analysis channels.

FIG. 12 is a plan view of an analysis cartridge having a convoluted storage channel, a plurality of reagent inlets, a convoluted mixing channel, a plurality of analysis regions, a plurality of valve and pump interfaces, and a waste storage channel.

FIG. 13, comprising FIGS. 13A-G, shows the individual sheets which are laminated together to form the analysis cartridge of FIG. 12.

FIG. 14 is a sample analysis instrument for use with a fluidic cartridge.

DETAILED DESCRIPTION OF THE INVENTION

This invention is further illustrated by the following preferred embodiments. In the drawings, like numbers refer to like features, and the same number appearing in more than one drawing refers to the same feature. The members of the flow systems of this invention are fluidically connected. The term "between" refers to the fluidic positioning, which does not necessarily correspond to the geometric positioning. The terms "top", "bottom" and "side" refer to the orientation in the drawings, which is not necessarily the orientation of the members in operation.

Figure 1 shows the flow system contained within the cartridge of this invention. The term cartridge is used herein for a fluidic device which is preferably, but not necessarily, disposable and which can be coupled with measurement, pumping, electronic, fluidic or other apparatus. It includes sample inlet **10**, convoluted sample storage channel **20**, resuspension pump interface **40**, sample analysis region **30** and valve interface **50**. The flow system is preferably a microfluidic flow system. The term microfluidic channel is used herein for fluid elements dimensioned so that flow therein is substantially laminar. In a laminar flow system turbulence is negligible. To maintain laminar flow in the storage channel, preferably the width of the channel is less than 2000 μm and the depth of the channel is less than 300 μm. To prevent clogging by particles, the dimension must be greater than the largest particle dimension, typically greater than 25 μm.

The sample inlet has an inlet shut-off interface to prevent the loaded sample from leaking out of the cartridge. In the illustrated embodiment the sample inlet comprises a

septum. A hypodermic needle is used to inject the sample through the septum. Upon removal of the needle, the septum forms a shut-off to keep the sample in the flow system. Alternatively, the sample inlet can be a non-sealing inlet such as a capillary or a channel which mates with a sample conduit. If the inlet does not have an integral shut-off interface, it can be combined with a separate valve interface.

The resuspension pump interface is used for reconstituting a sedimented sample following stop flow or storage. The pump can provide continuous or reversible flow. For continuous flow resuspension, the leading edge and trailing edge of the sample storage segment must be discarded, but the sample segment in the middle is resuspended to a homogeneous mixture identical to the original sample. Significant operating parameters are the resuspension flow rate and the resuspension time. Reversible flow resuspension uses a plurality of dispense/aspirate cycles. In this protocol, in each cycle the sedimented sample is swept through the channel in dispense mode and then swept back in aspirate mode. The swept volume is typically 1-4 periods of the spatially periodic channel. The aspirated volume is typically equal to the dispensed volume. The significant operating parameters are the resuspend swept volume, the number of resuspension cycles and the resuspension flow rate. For either protocol, the resuspension parameters are specific to the particle ladened fluid under consideration and the geometry of the storage channel. Suitable resuspension flow rates and times can be calculated or determined empirically.

To calculate the required flow rate, V, the channel geometry and fluid properties are considered. For substantially rectangular geometries, the critical flow rate is a function of the width W and depth D of the channel and of the effective viscosity μ_{eff} of the particulate suspension according to:

$$\dot{V} = \frac{2D^2 W \tau_{crit}}{3\mu_{eff}} \qquad \text{Equation 1}$$

By extrapolation of the data in Alonso et al. (1989), Biorheology **26**, 229-246, the critical wall shear stress, τ_{crit}, for cell suspension maintenance is estimated to be 0.14 Pa. As shown by Eq. 1, for greater channel dimensions the critical flow rate is greater. For a channel 50

μm x 100 μm in cross-section, the critical flow rate is 0.008 μl/s. For a 300 μm x 1000 μm channel, the critical flow rate is 2.8 μl/s.

The valves and pumps of this invention can be entirely incorporated in the cartridge, or the cartridge can include only valve and pump interfaces, and the remainder of the valve and pump mechanisms can be external to the cartridge. A pump (valve) comprises a pump (valve) interface and a pump (valve) mechanism. The interface is that portion which is directly connected to flow elements, and the mechanism is the exterior portion. The cartridge can be inserted in measurement apparatus comprising valve and pump mechanisms. Upon loading the cartridge in the apparatus, the valve and pump mechanisms engage the valve and pump interfaces. The valve can be either normally open or normally closed. They can be manually or automatically actuated.

Sedimentation in convoluted storage channels is illustrated in FIG. 2. When the flow is arrested the particles sediment in the nearest particle capture region, which are bends at gravitational potential minima. The gravity vector is illustrated in the drawings. The channels contain a plurality of particle capture regions so that the particles cannot aggregate in a single clump. The illustrated convoluted channels are spatially periodic. The term spatially periodic channel is used herein for a channel having a substantially constant number of particle capture regions per unit volume. This facilitates recreating a homogeneous sample upon resuspension. The illustrated embodiments are spatially periodic in a conventional geometric sense, having repeating units of length λ. Alternatively, the channel can be randomly convoluted but nonetheless have a substantially constant number of particle capture regions per unit volume.

The channel of FIG. 2A is suitable for storing particle-containing liquid in the illustrated orientation. If it were aligned along the channel axis, i.e. rotated so that the inlet and outlet were at the top, all of the particles would accumulate in the bottom capture region and would be difficult to resuspend uniformly. This type of spatially periodic channel is referred to herein as anisotropic because the suitability for storage depends on orientation. This anisotrophy can be disadvantageous. To prevent clumping the cartridge must be carefully handled to ensure that it is never aligned along the channel axis.

The channel of FIG. 2B can be used for storage at any orientation and is thus referred to herein as an isotropic storage channel. Isotropic channels are preferred because it is not necessary to maintain a particular orientation during handling. Further examples of isotropic spatially periodic channels are shown in FIG. 3. The channel of FIG. 3A has the same structure as the channel of FIG. 2B but with more repeated units. The channel of FIG. 3B is similar but with rounded corners. This can be advantageous for manufacturing and assembly. The channels of FIGS. 3C and D are referred to as "omega" channels, angular in FIG. 3C and rounded in FIG. 3D. Omega channels are similar to the square wave channel of Fig. 2A except that bringing the bases of the square wave toward one another adds additional capture regions, and thereby makes the channel isotropic. Figure 3 shows a few examples of storage channels; numerous other isotropic spatially periodic channels can be utilized. In the following schematic drawings square waves are used as a generic illustration of convoluted channels. Other embodiments may be preferred and in particular isotropic channels may be preferred.

This invention also provides a structure containing an isotropic storage channel. The structure is any solid material with a channel formed therein. The structure can be a disposable cartridge or a permanently installed element of a measurement or reaction instrument. It can be a microscale channel dimensioned for laminar flow or a macroscale channel dimensioned for turbulent flow. One embodiment is a bioreactor wherein reagents, which can include cells, are incubated in the channel followed by resuspension of particles.

A preferred embodiment of valve interface **50** is shown in FIG. 4. Figure 4A shows a cross-section of the valve in the open position and FIG. 4B shows the valve in the closed position. Channel **21**, running orthogonal to the plane of the paper, has walls formed by sheet **162B**, and top and bottom formed by sheets **162A** and **C**. Elastic seal **51** fits within an opening in sheet **162A**. The fluid element containing sheets are sandwiched between upper cartridge case **130** and lower cartridge case **131**. The valve mechanism includes valve pin **150** which is made of a rigid material, for example metal or plastic. The valve pin is guided by an opening in upper case **130**. When actuated, the pin presses against seal **51**, which extrudes into the channel, thereby closing it. Note that although it is termed a pinch valve, the channel itself is not pinched closed. The valve mechanism can be incorporated into the

cartridge or it can be a separate element. Seal **51** is made of a deformable material such as silicone, urethane, natural rubber or other elastomers. In the illustrated embodiment, the channel is formed with three separate sheets, **162 A-C**; it can instead be formed in fewer than or in more than three sheets. The pinch valve of FIG. 4 is an example of a valve that can be used with the analysis cartridge. Other valves can instead be used.

An embodiment of resuspension pump interface **40** is shown in cross-section in FIG. 5. Channel **22A**, running orthogonal to the plane of the paper, has walls formed within sheet **164B** and bottom formed by sheet **164C**. Fluid communication via **22** is a circular hole in sheet **164A** allowing fluid flow from **140** to **22A**. Elastic seal **41** fits between sheet **164A** and upper cartridge case **130**. The pump mechanism includes cannula **140**, which is preferably connected to a syringe pump, not shown. The cannula can be inserted into seal **41** to introduce fluids into channel **22A**. The cannula can be essentially a needle with a polished tip to avoid damaging the seal. In the resuspension procedure, a fluid such as saline or water is it injected into the channel through the cannula, and it sweeps the sample fluid through the channel. To reverse the flow, the saline in extracted through the cannula. The syringe pump interface can be used both as a pump, one- or two-directional, and as a reagent inlet. The entire pump, interface and mechanism, can be incorporated in the cartridge, or only the interface can be incorporated and the mechanism can be separate.

The sample analysis region provides for detection by any means known in the art, for example optical, electrical, pressure sensitive, or flow sensitive detection. More then one analysis means can be employed in a single analysis region, for example optical and electrical. For electrical detection, the cartridge can include an electrical interconnect. The cartridge can be electrically connected to electrical measuring apparatus. For optical detection, the cartridge can include a window positioned over the analysis region for optical coupling with measuring apparatus such as light sources and photodetectors. The windows can be inserted glass or, if the channel is formed in transparent sheets, the sheets themselves can serve as windows. The optical detection can be absorption, luminescent, fluorescent or scattering based. The cartridge can comprise a plurality of sample analysis regions. One of the analysis regions can provide a filling status gauge to indicate that the storage channel is filled. The gauge can be based on optical absorption measurement, pressure measurement,

conductivity measurement, flow measurement or any measurement that indicates the presence of a fluid in the gauge. For absorption measurement, visual observation of filling status may be used.

In a preferred embodiment, the analysis region is a flow cytometric analysis region. Preferably a sheath flow assembly is positioned along the analysis channel before the flow cytometric analysis region. Figures 6 and 7 illustrated a preferred embodiment of the sheath flow assembly. The assembly comprises seven sheets, **166A-G**, which are laminated together to form the fluidic elements of analysis cartridge **160**. The analysis channel, comprising core stream channel **26** and sheathed stream channel **27**, is connected to the convoluted storage channel (not shown). In sheath flow assembly **70**, first and second sheath fluid channels, jointly labeled as element **72**, are positioned on either side of and converge with channel **26**. In this embodiment the diameter of the sheathed portion is greater than the core portion of the analysis channel. The sheath fluid channels extend into layers **166C** and **E**, and are labeled as elements **75** and **76**. The sheath fluid channels provide hydrodynamic focusing of particles in channel **27** in the widthwise direction. Upper and lower sheath fluid chambers **73** and **74** are formed in sheets **166B** and **F**. When assembled, they are positioned above and below and converge with channel **26**. The sheath fluid chambers provide hydrodynamic focusing in the depthwise direction. To minimize layer to layer depthwise discontinuities in the region where the sheath fluid channels and chambers converge with the analysis channel, the downstream edges are staggered. The edge of channels **75** and **76** are slightly to the right of the edge of channel **72**. Sheath fluid is conducted to the sheath flow assembly through sheath fluid channel **71**. Vias **77** in sheets **166C-E** connect channel **71** with the sheath fluid chambers. The sheath fluid chambers communicate fluid to the sheath fluid channels. In typical hydrodynamic focusing operation, the ratio of sheath flow to core stream **26** flow is around 130:1.

Following hydrodynamic focusing, flow cytometric measuring is performed in analysis region **30**. The analysis region includes window recesses **31** and **32** in sheets **166C** and **E** positioned above and below the focused sample. The window recesses accommodate glass inserts. In lieu of recesses, sheets **166C** and **E** can themselves serve as windows. In the remaining sheets, optical clearing holes **33** allow optical access to the analysis region. The

sheets in FIG. 7 are sandwiched between an upper case and a lower case. Layers **166A** and **G** can be incorporated in the case. The illustrated embodiment also includes waste storage container **100**. It is connected with flow channel **23** through vias **101** and to a case mounted storage container through vias **102**.

One embodiment of the sheath flow assembly has been illustrated. Other sheath flow assemblies known in the art can be utilized, for example U.S.P.N. 4,983,038. Because this sheath flow assembly of the present invention provides both widthwise and depthwise hydrodynamic focusing, geometric focusing is not required. Although not necessary, the analysis channel can decrease in width and/or depth and in a downstream direction. Two-dimensional hydrodynamic focusing can also be achieved using the device of U.S. Patent Application 08/823,747, filed March 26, 1997. In lieu of hydrodynamic focusing the flow channel can be constricted in the analysis region to provide single file particles, as described in single file, as described in U.S. Patent No. 5,726,751.

Another preferred embodiment of the sample analysis region is an absorption analysis region. For increased sensitivity using an absorbance-based assay the optical pathlength, i.e. the channel depth, in the absorption measurement region is increased. For decreased sensitivity to factors such as intermittent sample stream perturbations, optical window quality and optical measurement apparatus lens defects, the effective illumination area of the detection region can be increased by increasing the channel width. There is a design trade-off between increasing the channel width and depth and minimizing the volume of the microfluidic system. This balance can be determined for a specific assay, a specific set of light sources, detectors and optics, and the required accuracy and resolution.

The cartridge can also include an inlet for mixing a reagent with the sample fluid prior to sample analysis, as shown in FIG. 8. The term "reagent" refers to any fluid that joins the sample fluid. It can be, for example, a diluent, a lysing agent, an indicator dye, a fluorescent compound, a fluorescent standard bead for flow cytometric calibration, or a reporter bead for flow cytometric measurement (U.S. Patent No. 5,747,349). Between storage channel **20** and analysis region **30**, reagent channel **80** joins analysis channel **24**. The reagent channel is connected to pump interface **40A** and reagent inlet **60**. In a preferred embodiment the pump

and the inlet are combined in a syringe pump. The cartridge includes valve interface **50** to separate the storage channel from the reagent inlet.

When the flow channels are microchannels having laminar flow therein, mixing between the reagent and the sample is predominantly diffusional mixing. The streams can join in side-by-side flow, as described in U.S. Patent No. 5,716,852 and U.S. Serial No. 08/829,679 filed March 31, 1997, or in a layered flow for more rapid mixing, as described in U.S. Serial No. 08/938,584 filed September 26, 1997 and U.S. Serial No. 08/938,585 filed September 26, 1997. In order to allow for mixing and reaction prior to analysis, a mixing channel can be included, as shown in FIG. 9. Mixing channel **90** is positioned between the reagent inlet and the analysis region. The geometry of mixing channel **90** is selected to allow mixing and reaction between the sample and reagent streams. The mixing channel can be convoluted in order to achieve the desired time delay within a compact space. Alternatively, active mixing methods can be employed, including ultrasonic, mechanical, sonic, flow induced, etc.

In the embodiment of FIG. 9 the mixing channel is illustrated as a square wave. For a particle-containing sample, it may be desired to allow diffusional mixing between smaller species within the sample and reagent streams without allowing particles in the sample screen to gravitationally settle into the reagent stream. Figure 10 shows the effect of channel geometry on gravitational mixing. A square wave channel is illustrated in FIG. 10A. The particle-containing sample stream enters mixing channel **90** through channel **24** and reagent stream enters through channel **80**. In the upper half of the mixing channel the sample stream is gravitationally above the reagent stream and particles tend to settle into the reagent stream. In the lower half of the mixing channel this is reversed and particles settle back into the sample stream. This reversal of top and bottom for the sample stream and reagents stream can be used more effectively in an isotropic channel as illustrated in FIG. 10B. In a spatially periodic isotropic channel the gravitational top and bottom of the channel interchange within each repeating unit. This counteracts the effect of gravity on the particles in the sample stream. The isotropic spatially periodic channel is therefore useful for sedimentation mitigation as well as sedimentation resuspension.

The cartridge can provide for more than one analysis region, in series or in parallel. Multiple parallel analysis regions are illustrated schematically in FIG. 11. The device of FIG. 11 comprises sample inlet **10**, storage channel **20**, resuspension pump interface **PI1** (Pump Interface 1), and analysis regions **30A-C**. At junctions **J1**, **J3**, **J5**, **J6** and at the end of the storage channel, fluid from the sample storage channel can be directed to analysis channels **24A-D** and to waste storage container **100**. Note that in this embodiment the resuspension pump is fluidically connected to the storage channel in the middle of the channel rather than at the beginning of the channel . Preferably the sample segment between **J1** and **J3** flows through valve **V3** for analysis, the sample segment between **J3** and **J5** flows through valve **V2** for analysis and the segment between **J5** and **J6** flows through valve **V1** for analysis.

The cartridge further includes pump interfaces **PI2-PI5**, valve interfaces **V1-V5**, reagent channels **80A-C**, sheath flow assembly **70**, waste storage container **100**, and vents **110A-C**. In a preferred embodiment, the sample inlet is a septum, the pump interfaces are syringe pump interfaces and the valve interfaces are pinch valve interfaces. The vents are made of gas permeable plugs having a reduced permeability when wet. The storage and mixing channels are illustrated as square waves but are preferably isotropic spatially periodic channels. The sheath flow assembly is preferably as illustrated in FIGS. 6 and 7. Analysis region **30C** is a filling status gauge providing visual indication of proper sample load. Analysis region **30A** is an absorption measurement region, optically coupled with measurement apparatus comprising both a green and a blue LED and a photodetector. Analysis region **30B** is a flow cytometric analysis region optically coupled with a measurement apparatus comprising a diode laser and a plurality of photodetectors at various optical axis and collection cone angles.

The cartridge of FIG. 11 can be used for hematology. A single cartridge can determine the red cell count, the total hemoglobin, and the white cell count and characterization. The analysis requires only 15 μl of sample, and the waste fluid is contained within the cartridge for safe operation and disposability. The sample is loaded into the storage channel through inlet **10**. At **J1** the potentially contaminated leading edge of the sample flows in bypass channel **25**, having a larger diameter than channel **20**. Air in the channel escapes through vent **110A**. The next segment of the sample fills the storage

channel. Valve **V4** is open and the sample flows to filling status indicator **30C**. Vent **110C** allows air to escape during sample loading. Excess sample flows into sample load bypass storage **115**. The cartridge can be stored or transported prior to analysis. For measurement the cartridge is inserted into a measurement instrument having a cartridge holder and valve and pump mechanisms, which engage the valve and pump interfaces on the cartridge. The pump mechanisms comprise syringe pumps wherein the syringes are filled with reagents. **P1** is filled with an inert driving fluid, **P2** is filled with diluent, **P3** is filled with a soft lysing agent, **P4** is filled with a Drabkin lysing reagent and **P5** is filled with a sheath fluid.

After insertion in the measurement apparatus, the sample is resuspended and analyzed. The entire measurement, including sample resuspension, can be performed in less than two minutes. The procedure for operating the analysis cartridge of FIG. 11 for hematology is outlined in Tables 1-3. For each time interval from t1 through t17, Table 1 describes the procedure, Table 2 gives the elapsed time, and Table 3 gives the status of valves and pumps fluidically connected to the cartridge and the status of optical measurement apparatus optically connected to the cartridge. In the first analysis time interval, t1, air is purged from resuspension pump interface **PI1** through valve **V5** into waste storage container **100**. In t2 the reagent and sheath fluid channels are purged and wet. In t3 the optical path in absorption measurement region **30A** is calibrated using the blue LED. In t4 the total hemoglobin sample segment between **J1** and **J3** is resuspended by alternating dispense and aspirate cycles using **P1**. In t5 the total hemoglobin assay is performed by mixing the blood with Drabkin reagent to lyse the red blood cells, and measuring the absorption in analysis region **30A**. To create a bubble-free mixture in the analysis region, air is purged from channels **24A** and **80A**. Preferably the sample fluid and the reagent reach **J2** simultaneously. Mixing channel **90A** is designed to allow formation of the cyanomethahemoglobin complex.

Following hemoglobin absorption assay, flow cytometric analysis is performed. In time intervals t6, t7 and t8 the channels used in flow cytometric analysis are purged. To protect optical surfaces in the cytometric region from direct contact with the sample, sheath fluid is pumped through the region during the purge. The sheath flow is set to a low ratio to minimize fluid accumulation in the waste storage container during priming stages. In t9 the RBC sample segment between **J5** and **J6** is resuspended. In t10 and t11 the optical

measuring apparatus is aligned and the flow is stabilized. In t12 and t13 the RBC flow cytometric assay is performed. In t14 the WBC sample segment between **J3** and **J5** is resuspended. In t15 a soft lysing reagent is added to the sample and time is allowed for mixing and reaction in mixing channel **90B**. In t16 and t17 the WBC assay is performed. The total elapsed time is 1.75 minutes. Following analysis, the cartridge is disposed of.

Drawings of a preferred embodiment of the hematology cartridge are shown in FIGS. 12 and 13. Figures 13A-G show the seven sheets, **167A-G**, which are laminated together to form cartridge **160** shown in FIG. 12. This is a three-dimensional fluidic structure wherein channels in different layers appear to overlap in FIG. 12 but are in fact separated by sheets **167C** and **E**. Vias in intervening sheets connect flow elements in different layers. Three-dimensional structures can be more compact and rugged than two-dimensional structures. Registry of the laminated sheets to the case is accomplished with holes **170** in the sheets. The case has pins that fit within holes **170**. For measurement, the cartridge is inserted into a measurement instrument including a cartridge holder. The outer case of the cartridge (not shown) has alignment markings thereon for optical and fluidic alignment with the measurement apparatus. In this embodiment, the alignment markings are kinematic alignment markings comprising a pit, a groove and a flat. The cartridge holder has corresponding pins. The shape of the cartridge is designed for engagement with the cartridge holder, and thus in itself comprises an alignment marking.

Sample is introduced through inlet **10** and stored in channel **20**. The sample leading edge flows into bypass channel **25**. The bypass channel is fluidically connected to a case-mounted waste storage container (not shown). Syringe pump interfaces **40A-E** and pinch valve interfaces **50A-D** control sample management in the cartridge. The syringe pump interfaces are also reagent inlets. When valve **50D** is open sample flows through channel **24D** to filling status gauge **30C**. For total hemoglobin assay lysing reagent is introduced through syringe pump interface **40D** and the mixture flows through analysis channel **24A** to absorption analysis region **30A**. For RBC assay, valve **50A** is opened, diluent is introduced through syringe pump interface **40B**, and the red blood cells are hydrodynamically focused in sheath flow assembly **70** and counted in flow cytometric analysis region **30B**. For WBC assay, valve **50B** is opened, a soft lysing agent, which masks red blood cells and platelets, is

introduced through syringe pump interface **40C**, mixing and reaction occur in mixing channel **90**, the sample is hydrodynamically focused in sheath flow assembly **70** and analyzed in flow cytometric analysis region **30B**. Waste fluid from all three analysis regions flows into waste storage container **100**, which is fluidically connected with a case-mounted storage container having a vent therein. This waste storage container is a channel. It can alternatively or in addition be a fixed or expandable reservoir.

In this embodiment, storage channel **20** and mixing channel **90** are formed in sheet **167D**. After cutting the sheet to form the channels, peninsulas of sheet material remain around the channels. The peninsulas are not well supported and can flop around during laminate assembly. A less floppy channel can be formed using two or more layers, with alternating loops of the channel formed in different layers.

The cartridge has been illustrated with particular mixing and measurement configurations. It can also provide filtering, diffusion based filtering as described in U.S. Serial No. 08/663,916 filed June 14, 1996, simultaneous particle separation and chemical reaction as described in U.S. Serial No. 08/938,585 filed September 26, 1997, valveless microswitching as described in U.S. Patent No. 5,726,404, diffusion-based chemical sensing as described in U.S. Patent No. 5,716,852, U.S. Serial No. 08/900,926 and U.S. Serial No. 08/936,093 and adsorption-enhanced differential extraction as described in U.S. Serial No. 08/876,038. The channel can also include fluidic elements for extraction, electrophoresis, electro-chemical reactions, chromatography and ion exchange reactions.

The cartridge can be fabricated from any moldable, machinable or etchable material. The term machining as used herein includes printing, stamping, cutting and laser ablating. The cartridge can be formed in a single sheet, in a pair of sheets sandwiched together, or in a plurality of sheets laminated together. The term "sheet" refers to any solid substrate, flexible or otherwise. The channels can be etched in a silicon substrate and covered with a cover sheet, which can be a transparent cover sheet. In a laminated embodiment, the channel walls are defined by removing material from a first sheet and the channel top and bottom are defined by laminating second and third sheets on either side of the first sheet . Any of the layers can contain fluid channels. In some cases the channel is simply a hole (or fluid via) to

route the fluid to the next fluid laminate layer. Any two adjacent laminate layers may be permanently bonded together to form a more complex single part. Often fluidic elements that have been illustrated in two separate layers can be formed in a single layer.

Each layer of a laminate assembly can be formed of a different material. The layers are preferably fabricated from substantially rigid materials. A substantially rigid material is inelastic, preferably having a modulus of elasticity less than 1,000,000 psi, and more preferably less than 600,000 psi. Substantially rigid materials can still exhibit dramatic flexibility when produced in thin films. Examples of substantially rigid plastics include cellulose acetate, polycarbonate, methylmethacrylate and polyester. Metals and metal alloys are also substantially rigid. Examples include steels, aluminum, copper, etc. Glasses, silicon and ceramics are also substantially rigid.

To create the fluidic element in the sheets, material is removed to define the desired structure. The sheets can be machine using a laser to ablate the material from the channels. The material can be removed by traditional die cutting methods. For some materials chemical etching can be used. Alternatively, the negative of the structure desired can be manufactured as a mold and the structure can be produced by injection molding, vacuum thermoforming, pressure-assisted thermoforming or coining techniques.

The individual layers, assemblies of layers, or molded equivalents are bonded together using adhesives or welding. Alternatively, mechanical compression through the use of fasteners such as screws, rivets and snap-together assembly can be used to seal adjacent layers. Layers can be assembled using adhesives in the following ways. A rigid contact adhesive (for example, 3M1151) can be used to join adjacent layers. A solvent release adhesive may be used to chemically bond two adjacent players. An ultraviolet curing adhesive (for example, Loctite 3107) can be used to join adjacent layers when at least one layer is transparent in the ultraviolet. Precision applied epoxies, thermoset adhesives, and thermoplastic adhesives can also be used. Dry coatings that can be activated to bond using solvents, heat or mechanical compression can be applied to one or both surfaces. Layers can be welded together. For welding the layers preferably have similar glass transition

temperatures and have mutual wetting and solubility characteristics. Layers can be welded using radio frequency dielectric heating, ultrasonic heating or local thermal heating.

The device of FIGS. 12 and 13 was fabricated as follows. Layers **167A** and **G** were made of 4 mil mylar and layers **167C** and **E** were made of 2 mil mylar. Layers **167B, D** and **F** were made of 2 mil mylar with 3M1151 on both sides (4 mil inclusive). The adhesive had cover sheets thereon. With the cover sheets still on the adhesive, the sheets were laser ablated to machine flow elements therein. The cover sheets were removed and the individual laminate was assembled with the aid of an alignment jig.

This invention further includes a sample analysis instrument for use with an analysis cartridge, in particular a hematology analysis cartridge. The instrument has a cartridge holder, a flow cytometric measuring apparatus position to be coupled with a flow cytometric measuring region on the cartridge, and a second measuring apparatus positioned to be coupled with a second measuring region on the cartridge. The flow cytometric measuring apparatus comprises a light source, preferably a laser, and at least one photodetector. The photodetectors can be positioned for measuring small angle scattering, large angle scattering or fluorescence. The apparatus can also include optical elements such as focusing and collection lenses, wavelength filters, dichroic mirrors and polarizers. The second measuring apparatus can be any measuring apparatus including optical, electrical, pressure sensitive and flow sensitive apparatus. Absorption measuring apparatus comprising a light source and a photodetector is preferred. Preferably the light source is positioned on a first side of the cartridge holder and the photodetector is positioned on the opposite side.

A measurement instrument is shown schematically in FIG. 14. It comprises cartridge holder **190**, flow cytometric measurement apparatus **180B** and absorption measurement apparatus **180A**. Cartridge **160**, shown in phantom, slides into the cartridge holder. The measurement apparati are positioned to be optically coupled with flow cytometric analysis region **30B** and absorption analysis region **30A**. This instrument also includes pump and valve mechanism manifold **141**. The pump mechanisms are syringe pumps which couple to pump interfaces on the cartridge via cannulas **140**. The manifold can also include reagent

reservoirs to refill the syringe pumps for multiple assays. The valve mechanisms activate valve pins **150**, which couple to valve interfaces on the cartridge.

Preferably the cartridge holder has alignment markings thereon to mate with corresponding markings on the cartridge. The alignment markings can be the shape of the holder, protruding pins, notches, rods, kinematic mounts, two stage kinematic mounts as described in U.S. Patent Application 08/736,336, filed October 23, 1996, or any other feature that facilitates positioning of the cartridge. In lieu of or in addition to cartridge alignment, the instrument can include optical steering elements, such as mirrors, to align the measuring apparatus with the analysis region. The analysis instrument can further include valve and pump mechanisms which couple with valve and pump interfaces on the cartridge.

All references cited herein are incorporated by reference in their entirety.

Preferred embodiments described above are intended to be illustrative of the spirit of this invention. Numerous variations and applications will be readily apparent to those skilled in the art. The range and scope of this patent is defined by the following claims.

Table 1

Time Interval Description

t1	Purge air from PI1 through valve V5.
t2	Purge air and wet delivery lines from PI2 to J7; PI3 to J7; PI4 to J2; and PI5 to J8
t3	THB optical path calibration using 430nm blue LED and Drabkin reagent absorption.
t4	THB Sample segment resuspension
t5	Total hemoglobin assay; purge of air from J1 to J2 & uniform mixing of sample + Drabkin & creation of a bubble free mixture in flow cell. Time allowed for the creation of the Cyanomethahemoglobin complex.
t6	RBC sample segment mis/air purge from J6 through J9&J7 to J8: Sheath pump is set to a low ratio, about 5:1 in order to protect optical surfaces of the cytometer section.
t7	WBC sample segment mis/air purge from J3 through J4&J7 to J8. Sheath pump is set to a low ratio, about 5:1 in order to protect optical surfaces of the cytometer section.
t8	J7 junction purge. Purge air from the region around J7 through the cytometer to waste.
t9	RBC sample segment resuspension
t10	Beam steering/optical targeting.
t11	RBC assay flow stabilization algorithm based on mean pulse frequency PID feedback control
t12	RBC assay.
t13	Second RBC assay (if required)
t14	WBC sample segment resuspension
t15	WBC assay flow stabilization and 15 second time delay.
t16	WBC assay.
t17	Second WBC assay (if required)

Table 2

Time interval	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	t17
Interval time(s)	1	3	2	3	10	2	2	1	3	5	4	4	3	1.6	17	22	22
Elapsed time(s)	1	4	6	9	19	21	23	24	27	32	36	40	43	45	62	83	105
Elapsed time (min)	0.02	0.07	0.10	0.15	0.32	0.35	0.38	0.40	0.45	0.53	0.60	0.67	0.72	0.74	1.03	1.39	1.75

Table 3
Resource Status

Time interval	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	t17
Resuspension pump, P1D (dispense)	X			X	X	X	X		X	X	X	X	X	X	X	X	X
Resuspension pump, P1A (asperiate)	X			X	X	X	X		X	X	X	X	X	X	X	X	X
Diluent pump, P2		X				X		X	X	X	X	X	X	X			
Soft Lyse pump, P3		X					X	X							X	X	X
THB pump, P4		X	X	X	X												
Sheath pump, P5		X				X	X	X	X	X	X	X	X	X	X	X	X
RBC Valve, V1	C[1]	O	C	C	C	O	C	O	O	O	O	O	O	O	C	C	C
WBC Valve, V2	C	O	C	C	C	C	O	O	C	C	C	C	C	C	O	O	O
THB Valve, V3	C	O	C	O	O	C	C	C	C	C	C	C	C	C	C	C	C
Waste Isolation Valve, V4	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Sample delivery purge, V5	O	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Beam Steering Motor, M1										X							
Beam Steering Motor, M2										X							
Diode laser										X	X	X	X	X	X	X	X
Green LED					X												
Blue LED			X														

[1] C = Closed, O = Open

We claim:

1. A fluidic sample analysis cartridge for analyzing a particle-containing liquid sample, comprising:

a sample inlet having an inlet shut-off interface;

a convoluted sample storage channel in fluidic connection with said inlet;

a resuspension pump interface in fluidic connection with said storage channel;

a first analysis channel in fluidic connection with said storage channel and having a first analysis region; and

a first analysis valve interface positioned between said storage channel and said first analysis region.

2. The cartridge of claim 1 wherein said storage channel is a spatially periodic channel.

3. The cartridge of claim 2 wherein said storage channel is an isotropic spatially periodic channel.

4. The cartridge of claim 2 wherein the width of said storage channel is between about 25 and 2,000 μm.

5. The cartridge of claim 4 wherein the depth of said storage channel is less than about 300 μm.

6. The cartridge of claim 1 wherein said resuspension pump interface is positioned between said sample inlet and said storage channel.

7. The cartridge of claim 1 wherein said resuspension pump interface is positioned along said storage channel.

8. The cartridge of claim 1 wherein said resuspension pump interface is a syringe pump interface.

9. The cartridge of claim 1 wherein said sample inlet comprises a septum.

10. The cartridge of claim 1 wherein said sample inlet comprises a valve interface.

11. The cartridge of claim 1 wherein said first analysis valve interface comprises a pinch valve interface.

12. The cartridge of claim 1 wherein said first analysis region comprises an electrical analysis region.

13. The cartridge of claim 12 wherein said electrical analysis region comprises an electrical interconnect.

14. The cartridge of claim 1 wherein said first analysis region comprises an optical analysis region.

15. The cartridge of claim 14 wherein said optical analysis region comprises a window.

16. The cartridge of claim 14 further comprising a sheath flow assembly positioned along said first analysis channel between said storage channel and said first analysis region.

17. The cartridge of claim 16 wherein said sheath flow assembly comprises first and second sheath fluid channels on either side of and converging with said first analysis channel.

18. The cartridge of claim 17 wherein the width of said first analysis channel does not contract within said sheath flow assembly.

19. The cartridge of claim 17 wherein said sheath flow assembly further comprises upper and lower sheath fluid chambers positioned above and below and converging with said first analysis channel.

20. The cartridge of claim 19 wherein said sheath flow assembly provides hydrodynamic focusing in both the widthwise and depthwise directions.

21. The cartridge of claim 17 wherein said analysis channel contracts in the widthwise and/or depthwise direction after converging with said sheath flow channels.

22. The cartridge of claim 1 further comprising a reagent inlet in fluid communication with said first analysis channel between said storage channel and said first analysis region.

23. The cartridge of claim 22 wherein said reagent inlet comprises a syringe pump interface.

24. The cartridge of claim 22 further comprising a reagent storage reservoir in fluid communication with said reagent inlet.

25. The cartridge of claim 22 further comprising a mixing channel between said reagent inlet and said first analysis region.

26. The cartridge of claim 25 wherein said mixing channel is a spatially periodic channel.

27. The cartridge of claim 26 wherein said mixing channel is an isotropic spatially periodic channel.

28. The cartridge of claim 1 wherein said first analysis channel further comprises a second analysis region, in series with said first analysis region.

29. The cartridge of claim 1 further comprising a second analysis channel, having a second sample analysis region, in parallel with said first analysis channel.

30. The cartridge of claim 29 wherein said first sample analysis region comprises a filling status gauge.

31. The cartridge of claim 1 further comprising a waste storage container fluidically connected with said first analysis channel.

32. The cartridge of claim 31 wherein said waste storage container comprises a waste storage channel.

33. The cartridge of claim 31 wherein said waste storage container is an expandable compartment.

34. The cartridge of claim 1 further comprising a vent in gaseous communication with said first analysis channel.

35. The cartridge of claim 34 wherein said vent is a gas-permeable plug, said plug having reduced permeability when in contact with a liquid.

36. The cartridge of claim 1 for use with a measurement apparatus, further including alignment markings for positioning said cartridge within said measurement apparatus.

37. The cartridge of claim 1 wherein said cartridge is made of three or more laminated sheets.

38. The cartridge of claim 37 wherein said laminated sheets are made of plastic.

39. The cartridge of claim 37 wherein said sheets are bonded together by adhesive substantially covering the abutting surfaces thereof.

40. A disposable fluidic hematology cartridge for analyzing a particle-containing liquid sample, comprising:

a sample inlet;

an absorption measuring channel fluidically coupled with said sample inlet and having an absorption measuring region;

a first valve interface positioned between said sample inlet and said absorption measuring region;

a flow cytometric measuring channel fluidically coupled with said sample inlet and having a flow cytometric measuring region; and

a second valve interface positioned between said sample inlet and said flow cytometric measuring region..

41. The hematology cartridge of claim 40 wherein said absorption measuring channel and said flow cytometric measuring channel are positioned in parallel.

42. The hematology cartridge of claim 40 wherein said absorption measuring and said flow cytometric measuring regions each comprise a first transparent window positioned over said measuring channels.

43. The hematology cartridge of claim 42 wherein said absorption measuring and said flow cytometric measuring regions each further comprise a second transparent window, positioned under said measuring channels.

44. The hematology cartridge of claim 42 wherein the optical pathlength of said absorption measuring channel is increased in said absorption measuring region.

45. The hematology cartridge of claim 44 wherein the width of said absorption measuring channel is increased in said absorption measuring region.

46. The hematology cartridge of claim 40 wherein said flow cytometric measuring channel is narrowed in said flow cytometric measuring region to constrict particles into single file.

47. The hematology cartridge of claim 40 further comprising a sheath flow assembly positioned along said flow cytometric measuring channel before said flow cytometric measuring region.

48. The hematology cartridge of claim 47 wherein said sheath flow assembly comprises first and second sheath flow channels on either side of and converging with said flow cytometric measuring channel.

49. The hematology cartridge of claim 48 wherein the width of said flow cytometric measuring channel does not contract within said sheath flow assembly.

50. The hematology cartridge of claim 48 wherein said sheath flow assembly further comprises upper and lower sheath fluid chambers positioned above and below and converging with said flow cytometric measuring channel.

51. The hematology cartridge of claim 40 further comprising a convoluted sample storage channel positioned before said flow cytometric measuring channel.

52. The hematology cartridge of claim 51 wherein said storage channel is a spatially periodic channel.

53. The hematology cartridge of claim 52 wherein said storage channel is an isotropic spatially periodic channel.

54. The hematology cartridge of claim 51 wherein said first valve interface is positioned between said storage channel and said absorption measuring region and said second valve interface is positioned between said storage channel and said flow of cytometric measuring region.

55. The hematology cartridge of claim 40 further comprising a first reagent inlet, positioned along said absorption measuring channel before said absorption measuring region.

56. The hematology cartridge of claim 55 further comprising a second reagent inlet, positioned along said flow cytometric measuring channel before said flow cytometric measuring region.

57. The hematology cartridge of claim 56 further comprising a sheath flow assembly positioned along said flow cytometric measuring channel between said second reagent inlet and said flow cytometric measuring region.

58. The hematology cartridge of claim 56 wherein each of said first and second reagent inlets comprises a syringe pump interface.

59. The hematology cartridge of claim 56 further comprising a mixing channel positioned along said flow cytometric measuring channel between said second reagent inlet and said flow cytometric measuring region.

60. The hematology cartridge of claim 59 wherein said mixing channel is a spatially periodic channel.

61. The hematology cartridge of claim 40 further comprising a waste storage container positioned downstream of said flow cytometric measuring region.

62. A method of blood analysis using the hematology cartridge of claim 40, comprising the steps of:

introducing a sample of blood into said sample inlet;

measuring the absorption of said blood in said absorption measuring region; and

measuring the scattering by said blood in said flow cytometric measuring region.

63. The method of blood analysis of claim 62 wherein said hematology cartridge further comprises a sheath flow assembly positioned along said flow cytometric measuring channel before said flow cytometric measuring region, and wherein said method further comprises the step of using said sheath flow assembly to hydrodynamically focus said blood.

64. The method of blood analysis of claim 62 wherein said hematology cartridge further comprises a convoluted sample storage channel positioned before said flow cytometric measuring region, and wherein said method further comprises the step of storing said blood in said storage channel, whereby particles in said blood sediment in said storage channel.

65. The method of blood analysis of claim 64 further comprising the step of resuspending said particles in said blood.

66. The method of blood analysis of claim 62 wherein said hematology cartridge further comprises a first reagent inlet positioned between said sample inlet and said absorption measuring region, and wherein said method further comprises the steps of introducing a cell lysing agent through said first reagent inlet and obtaining the hemoglobin content of said blood from the measured absorption.

67. The method of blood analysis of claim 66 wherein said hematology cartridge further comprises a second reagent inlet positioned between said sample inlet and said flow cytometric measuring region, and wherein said method further comprises the steps of introducing a second reagent through said second reagent inlet and characterizing the white blood cells from the measured scattering.

68. The method of blood analysis of claim 67 wherein said second reagent masks red blood cells and platelets.

69. The method of blood analysis of claim 67 wherein said hematology cartridge further comprises a mixing channel positioned between said second reagent inlet and said flow cytometric measuring region, and wherein said method further comprises the step of allowing said blood and said second reagent to mix and react in said mixing channel.

70. The method of blood analysis of claim 62 wherein said hematology cartridge further comprises a waste storage container positioned downstream of said flow cytometric measuring region, and wherein said method further comprises the step of collecting said blood in said waste storage container.

71. The method of blood analysis of claim 70 further comprising the step of disposing of said hematology cartridge after use.

72. A method of storing a particle-containing liquid, comprising the steps of:

flowing the liquid into a convoluted storage channel having a plurality of particle capture regions; and

arresting the liquid in said channel, thereby allowing the particles to sediment within the particle capture regions of said storage channel.

73. The method of claim 72 wherein said storage channel is a spatially periodic channel.

74. The method of claim 73 wherein said storage channel is an isotropic spatially periodic channel and wherein said step of arresting the liquid does not include the step of orienting said channel in a preferred direction.

75. The method of claim 72 further including the step of resuspending said particles in said liquid.

76. The method of claim 75 wherein said step of resuspending comprises the step of flowing a resuspension fluid through said storage channel, whereby said sample is swept out of said storage channel.

77. The method of claim 76 wherein said step of resuspending further comprises the step of discarding the leading edge of said sample swept out of said storage channel.

78. The method of claim 75 wherein said step of resuspending comprises at least one dispense/aspirate cycle, a cycle comprising the steps of sweeping said sample in a first direction through a portion of said storage channel and drawing said sample in the reverse direction through a portion of said storage channel.

79. The method of claim 78 wherein said step of resuspending comprises a plurality of dispense/aspirate cycles.

80. A sedimentation mitigation structure comprising an isotropic spatially periodic channel.

81. The sedimentation mitigation structure of claim 80 wherein said channel is a microscale channel.

82. The sedimentation mitigation structure of claim 81 wherein said storage channel is a macroscale channel.

83. The sedimentation mitigation structure of claim 80 further comprising a pump interface fluidically connected to said channel.

84. The sedimentation mitigation structure of claim 83 further comprising a valve interface fluidically connected to said channel.

85. The sedimentation mitigation structure of claim 80 wherein said channel is a three-dimensional channel.

86. The sedimentation mitigation structure of claim 85 wherein said cartridge is made of three or more laminated sheets.

87. The sedimentation mitigation structure of claim 86 wherein a first portion of said channel is formed between a first and a second laminated sheet and wherein a second portion

of said channel, fluidically connected to said first portion of said channel, is formed between said second and a third laminated sheet.

88. A sample analysis instrument for use with a fluidic cartridge, said cartridge containing a liquid sample and having first and second analysis regions, said apparatus comprising:

a cartridge holder;

a flow cytometric measuring apparatus positioned to be optically coupled with said first analysis region; and

a second measuring apparatus positioned to be coupled with said second analysis region.

89. The instrument of claim 88 wherein said flow cytometric measuring apparatus comprises a light source aligned to illuminate said first analysis region and further comprises a first photodetector aligned to collect scattered light from said first analysis region.

90. The instrument of claim 89 wherein said flow cytometric measuring apparatus further comprises a second photodetector aligned to collect scattered or fluorescent light from said first analysis region.

91. The instrument of claim 88 wherein said second measuring apparatus comprises absorption measuring apparatus.

92. The instrument of claim 88 wherein said second measuring apparatus comprises electrical measuring apparatus.

93. The instrument of claim 88 wherein said cartridge has cartridge alignment markings thereon and wherein said holder has alignment markings thereon to mate with said cartridge alignment markings.

94. The instrument of claim 88 wherein said cartridge contains a pump interface and wherein said apparatus further comprises a pump mechanism positioned to couple with said pump interface.

95. The instrument of claim 94 wherein said pump interface is a syringe pump interface and wherein said pump mechanism is a syringe pump.

96. The instrument of claim 88 wherein said cartridge contains a valve interface and wherein said apparatus further comprises a valve mechanism positioned to couple with said valve interface.

97. The instrument of claim 96 wherein said valve interface is a pinch valve interface and wherein said valve mechanism is a pinch valve mechanism.

98. A fluidic cartridge for analyzing a particle-containing sample, comprising:

a sample inlet;

a sample storage container in fluidic communication with said sample inlet;

a first sample analysis region in fluidic communication with said sample storage container;

a first sample analysis valve interface positioned between said storage container and said first analysis region; and

a resuspension means for resuspending particles sedimented in said sample storage container.

99. The cartridge of claim 98 wherein said sample storage container comprises a convoluted sample storage channel and wherein said resuspension means comprises a resuspension pump interface.

100. The cartridge of claim 99 wherein said resuspension pump interface is a syringe pump interface.

101. The cartridge of claim 98 wherein said sample storage container comprises a reservoir and wherein said resuspension means comprises an ultrasonic vibrator acoustically coupled to said reservoir.

102. The cartridge of claim 98 wherein said sample storage container comprises a reservoir and wherein said resuspension means comprises a mechanical agitator positioned within said reservoir.

103. The cartridge of claim 102 wherein said mechanical agitator comprises a stir bar.

104. The cartridge of claim 102 wherein said mechanical agitator comprises a piston.

105. The cartridge of claim 98 wherein said sample storage compartment comprises a reservoir, and wherein said resuspension means comprises a mechanical agitator positioned outside of said reservoir and vibrationally coupled with said reservoir.

106. A method of fabricating a laminated fluidic flow cartridge, comprising the steps of:

providing a plurality of rigid sheets, each sheet having flow elements formed therein;

stacking said rigid sheets; and

bonding abutting surfaces of said rigid sheets.

107. The method of claim 106 wherein said step of providing said rigid sheets having flow elements formed therein comprises the steps of:

providing a plurality of rigid sheets; and

machining flow elements in said rigid sheets.

108. The method of claim 107 wherein said step of machining is selected from the group consisting of laser ablating and die cutting.

109. The method of claim 106 wherein said step of providing said rigid sheets having flow elements formed therein is selected from the group consisting of injection molding, vacuum thermoforming, pressure-assisted thermoforming and coining.

110. The method of claim 106 wherein said rigid sheets are selected from the group consisting of cellulose acetate, polycarbonate, methylmethacrylate and polyester.

111. The method of claim 106 wherein said step of bonding abutting surfaces of said rigid sheets uses an adhesive.

112. The method of claim 111 wherein said adhesive is selected from the group consisting of rigid contact adhesive, solvent release adhesive, ultraviolet curing adhesive, epoxy, thermoset adhesive, thermoplastic adhesive and dry coating adhesive.

113. The method of claim 106 wherein said step of bonding abutting surfaces of said rigid sheets comprises welding said sheets together.

114. The method of claim 113 wherein said welding uses a method selected from the group consisting of radio frequency dielectric heating, ultrasonic heating and local thermal heating.

115. The method of claim 106 wherein at least alternate layers of said rigid sheets comprise rigid sheets coated with rigid contact adhesive and wherein said step of providing said rigid sheets having flow elements formed therein comprises the step of machining said flow elements in said rigid sheets by laser ablation.

116. The method of claim 115 wherein said rigid sheets coated with rigid contact adhesive are further coated with cover sheets and wherein said method further comprises the step of

removing said cover sheets after said laser ablation and prior to said step of stacking said rigid sheets.

117. The method of claim 116 wherein said rigid sheets are polyester sheets.

118. A sheath flow assembly comprising:

a sample flow channel;

a first and a second sheath fluid channel positioned on either side of and converging with said sample flow channel; and

an upper and a lower sheath fluid chamber positioned above and below and converging with said sample flow channel.

119. The sheath flow assembly of claim 118 wherein said first and second sheath fluid channels and said upper and lower sheath fluid chambers simultaneously converge with said sample flow channel.

120. The sheath flow assembly of claim 118 wherein the width of said sample flow channel does not contract within said assembly.

121. The sheath flow assembly of claim 118 wherein said first and second sheath fluid channel
provide hydrodynamic focusing in a widthwise direction and said upper and lower sheath fluid chambers provide hydrodynamic focusing in a depthwise direction.

122. The sheath flow assembly of claim 118 wherein said assembly is fabricated from at least first second and third laminated sheets, and wherein the walls of said lower sheath fluid chamber are formed in said first sheet, the walls of said sample flow channel and said first and second sheath fluid channels are formed in said second sheet and the walls of said upper sheath fluid chamber are formed in said third sheet.

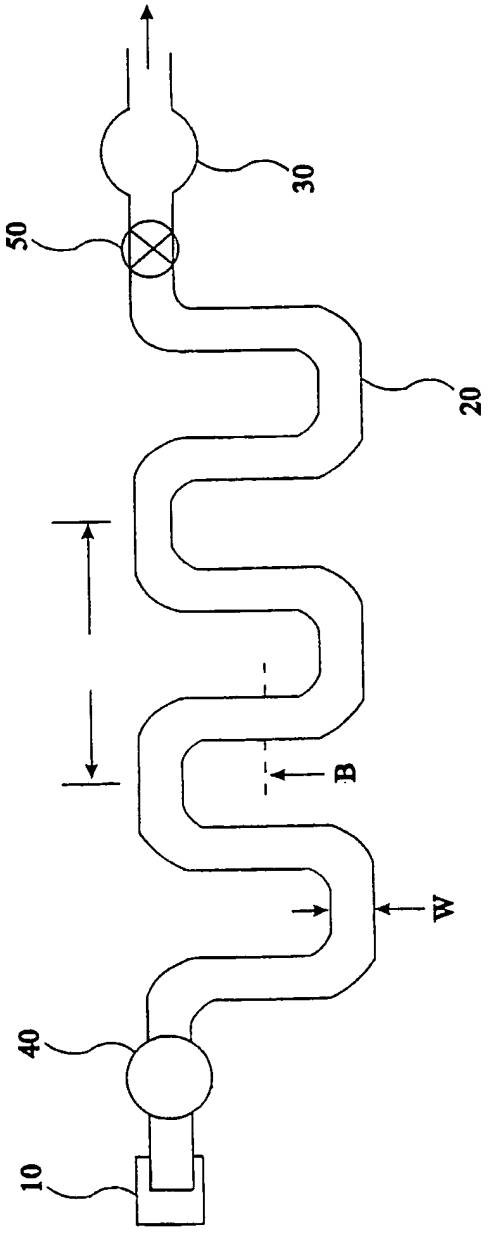


FIG. 1A

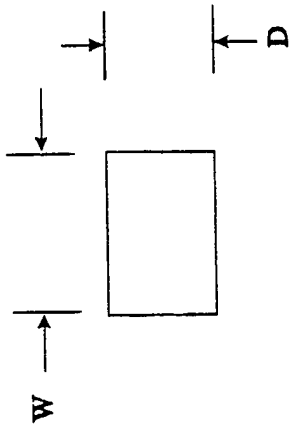


FIG. 1B

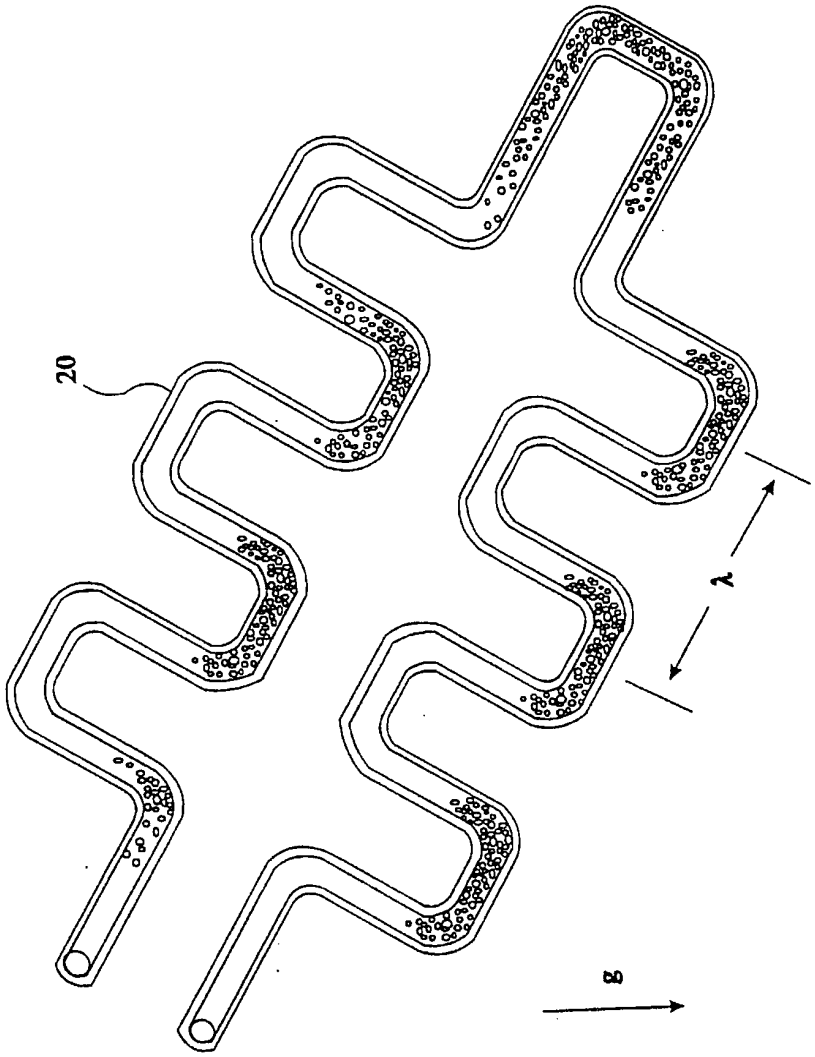


FIG. 2A

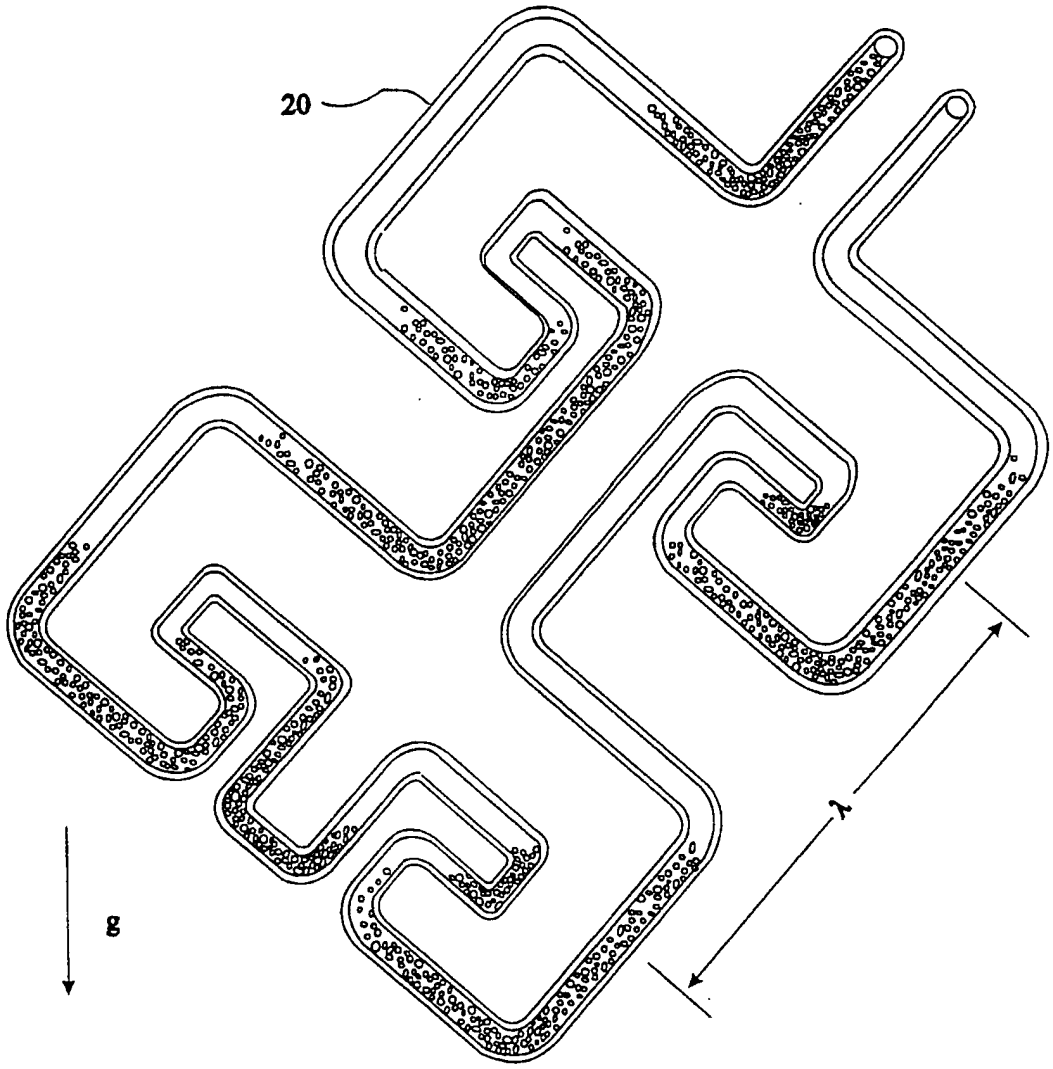


FIG. 2B

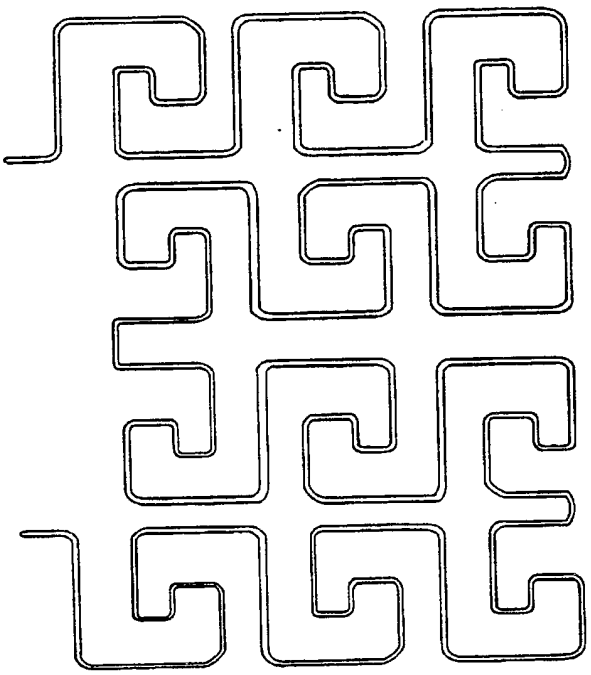


FIG. 3A

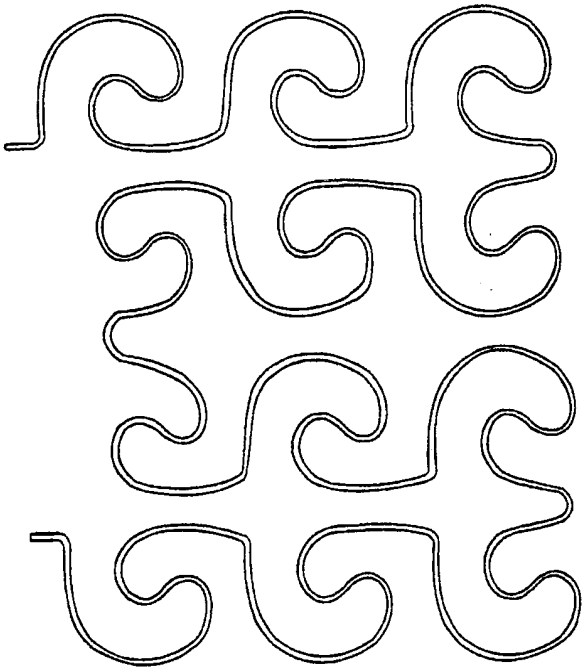


FIG. 3B

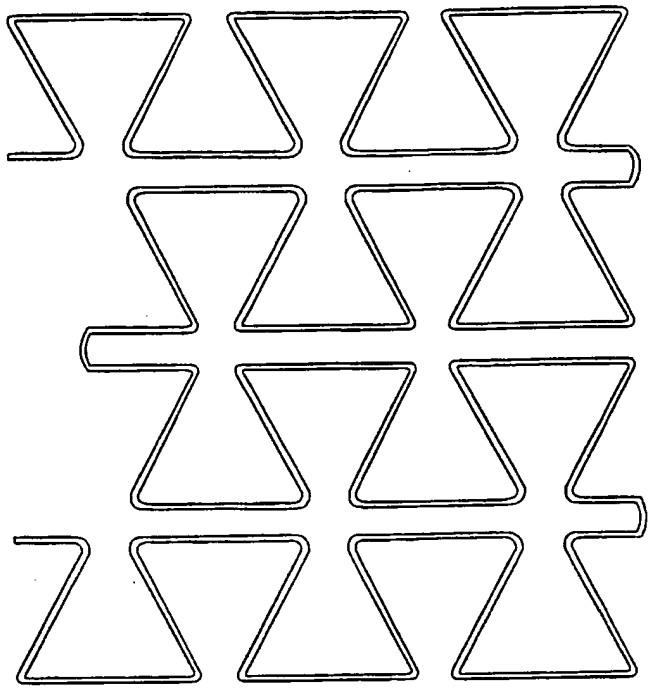


FIG. 3C

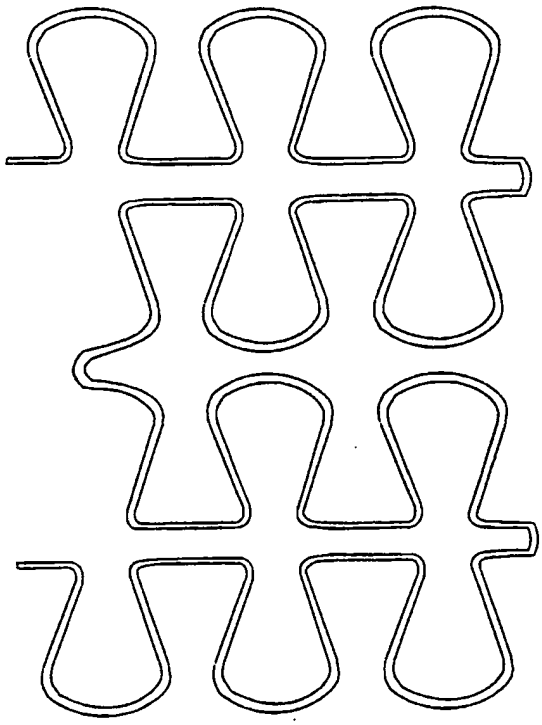


FIG. 3D

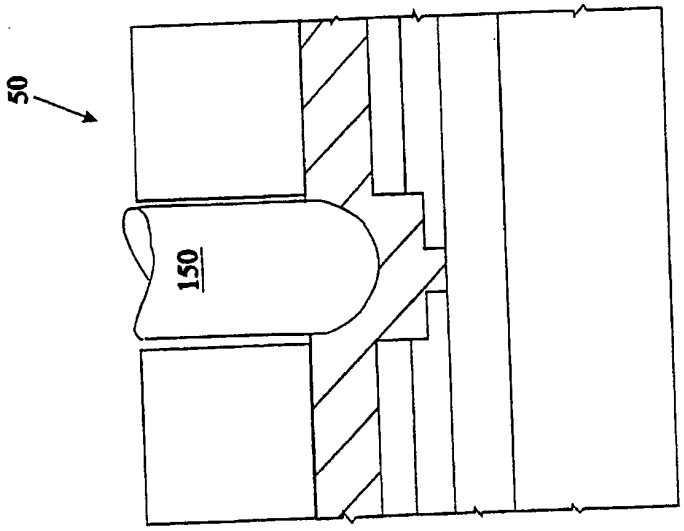


FIG. 4B

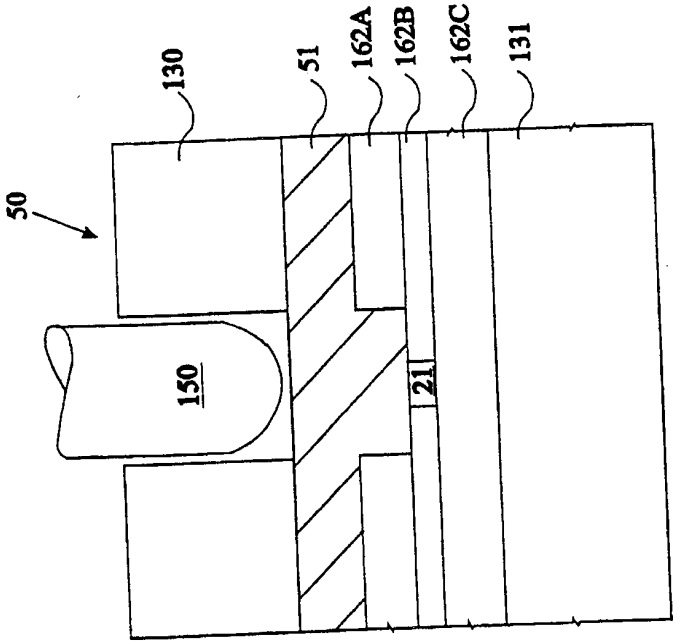


FIG. 4A

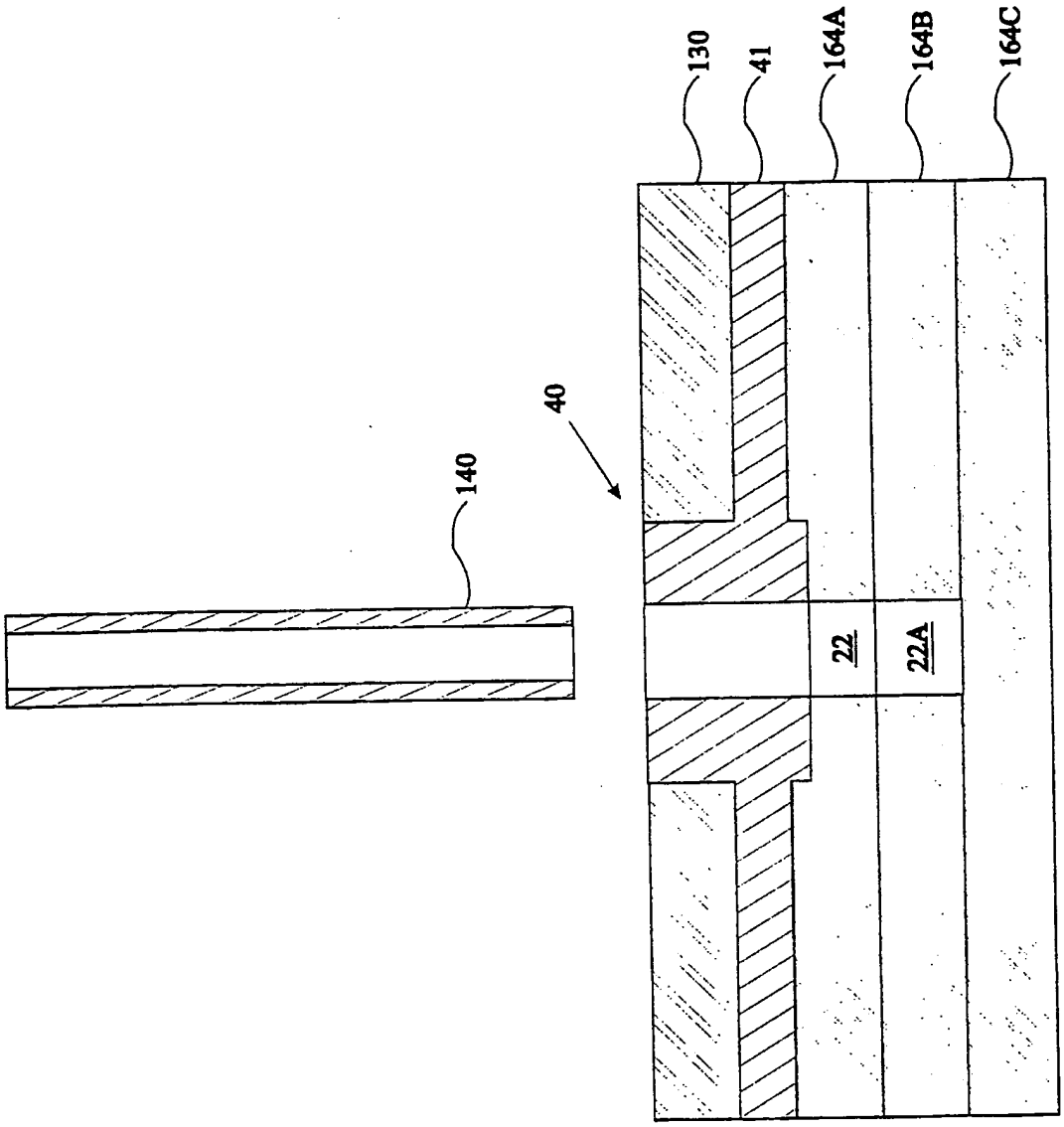


FIG. 5

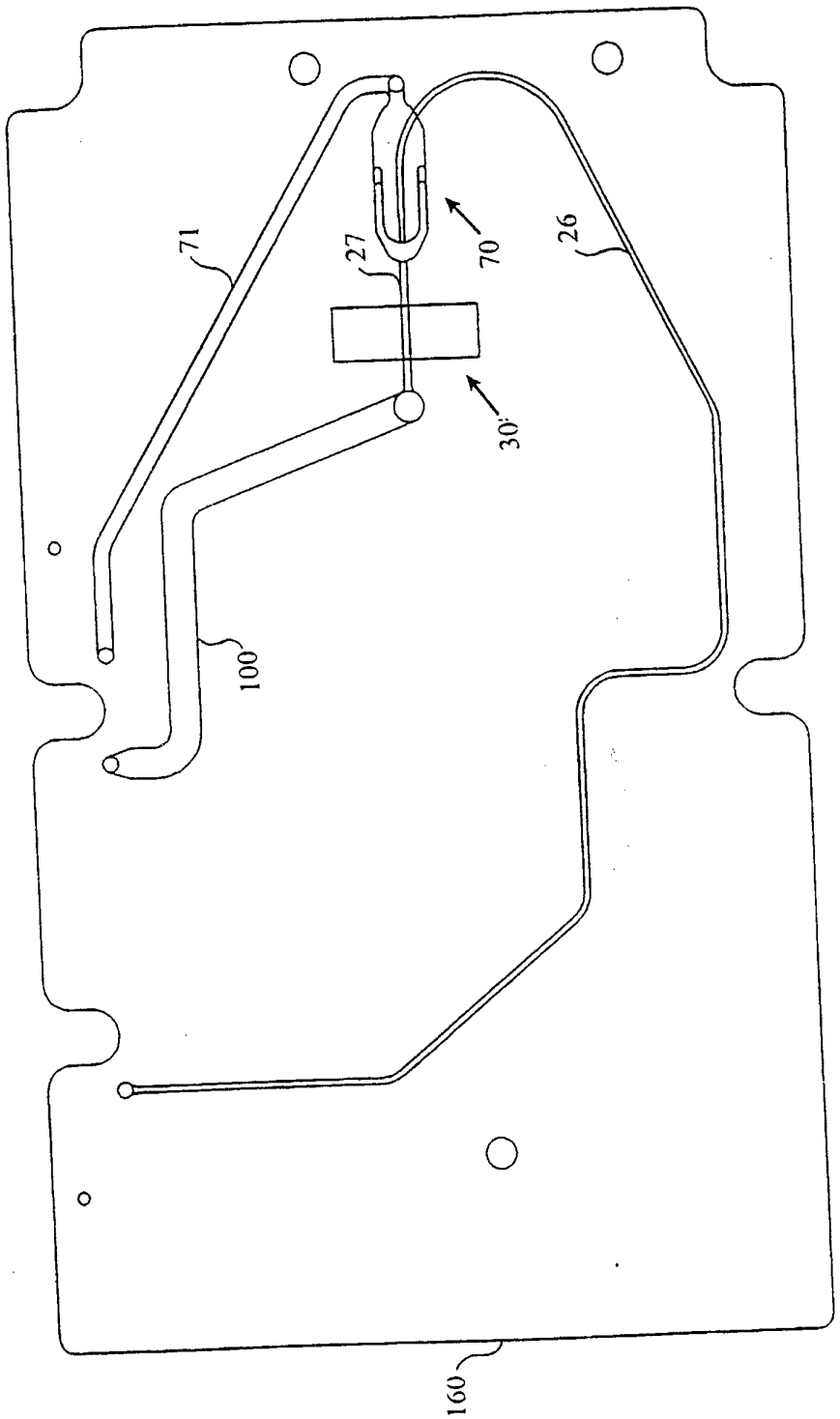


FIG. 6

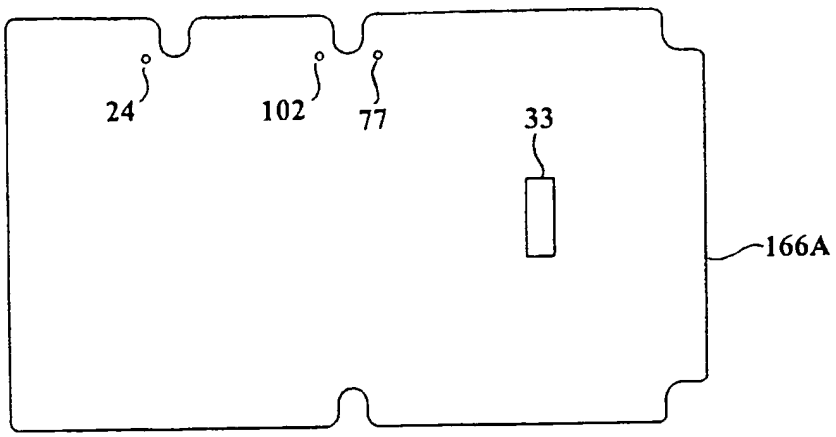


FIG. 7A

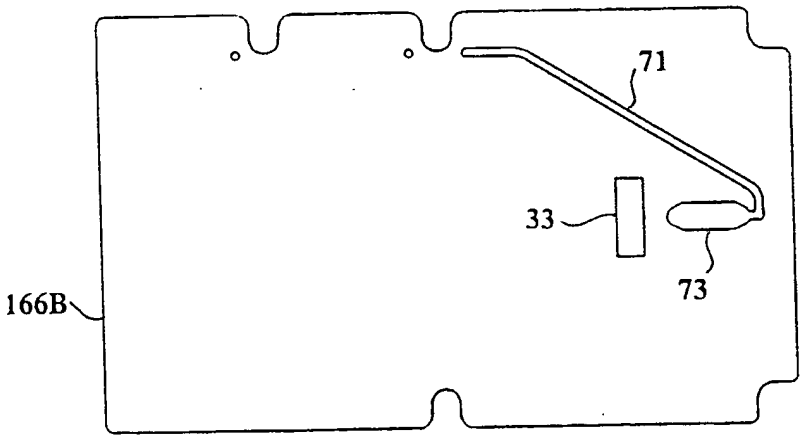


FIG. 7B

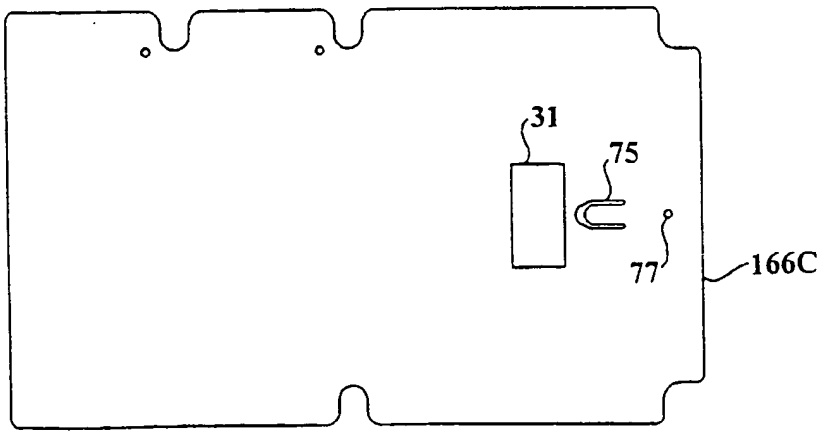


FIG. 7C

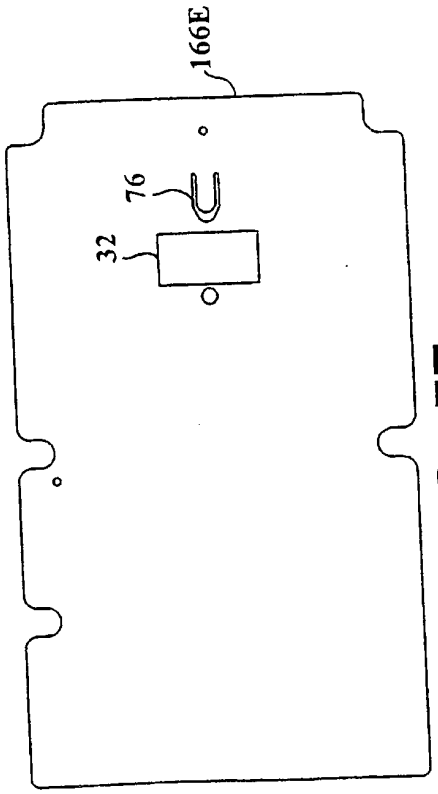


FIG. 7E

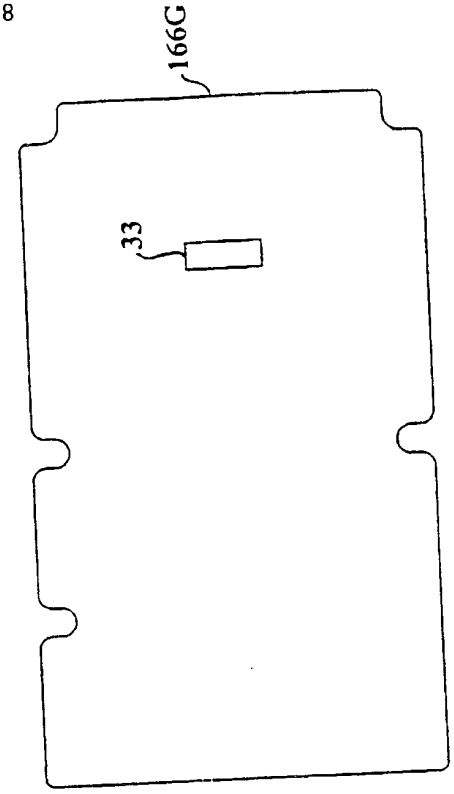


FIG. 7G

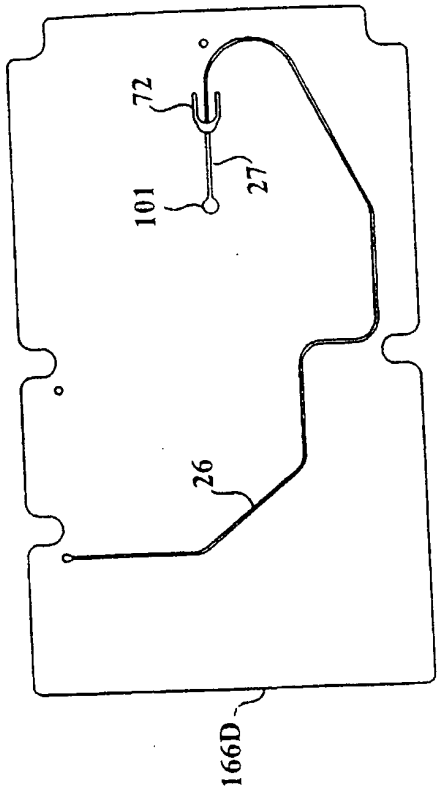


FIG. 7D

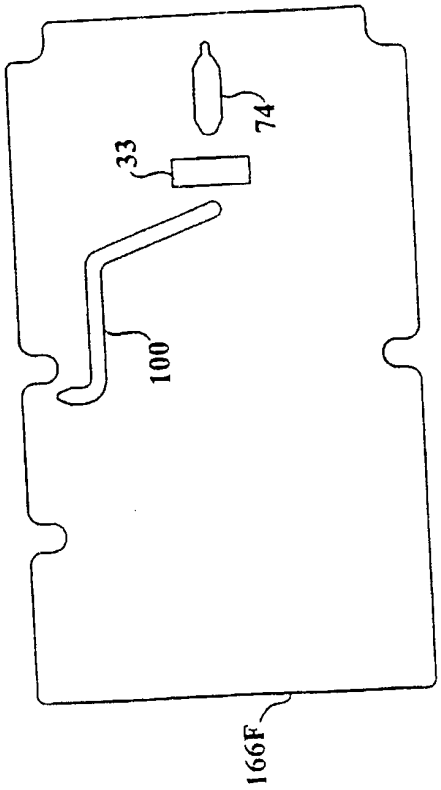


FIG. 7F

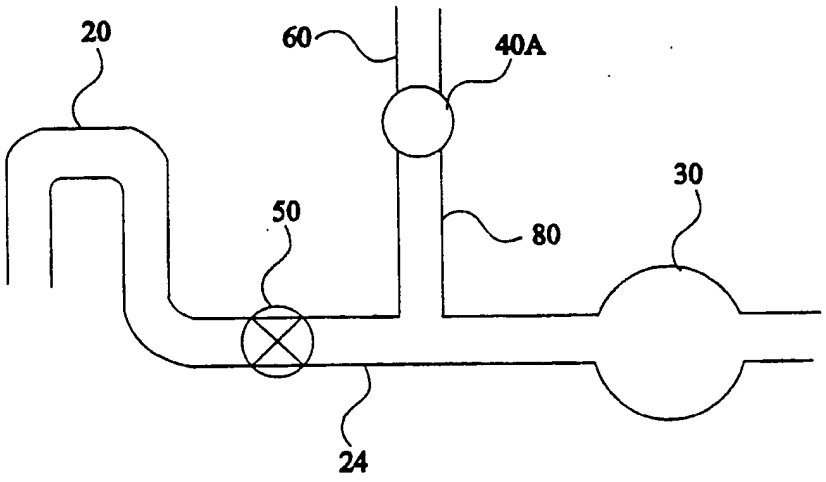


FIG. 8

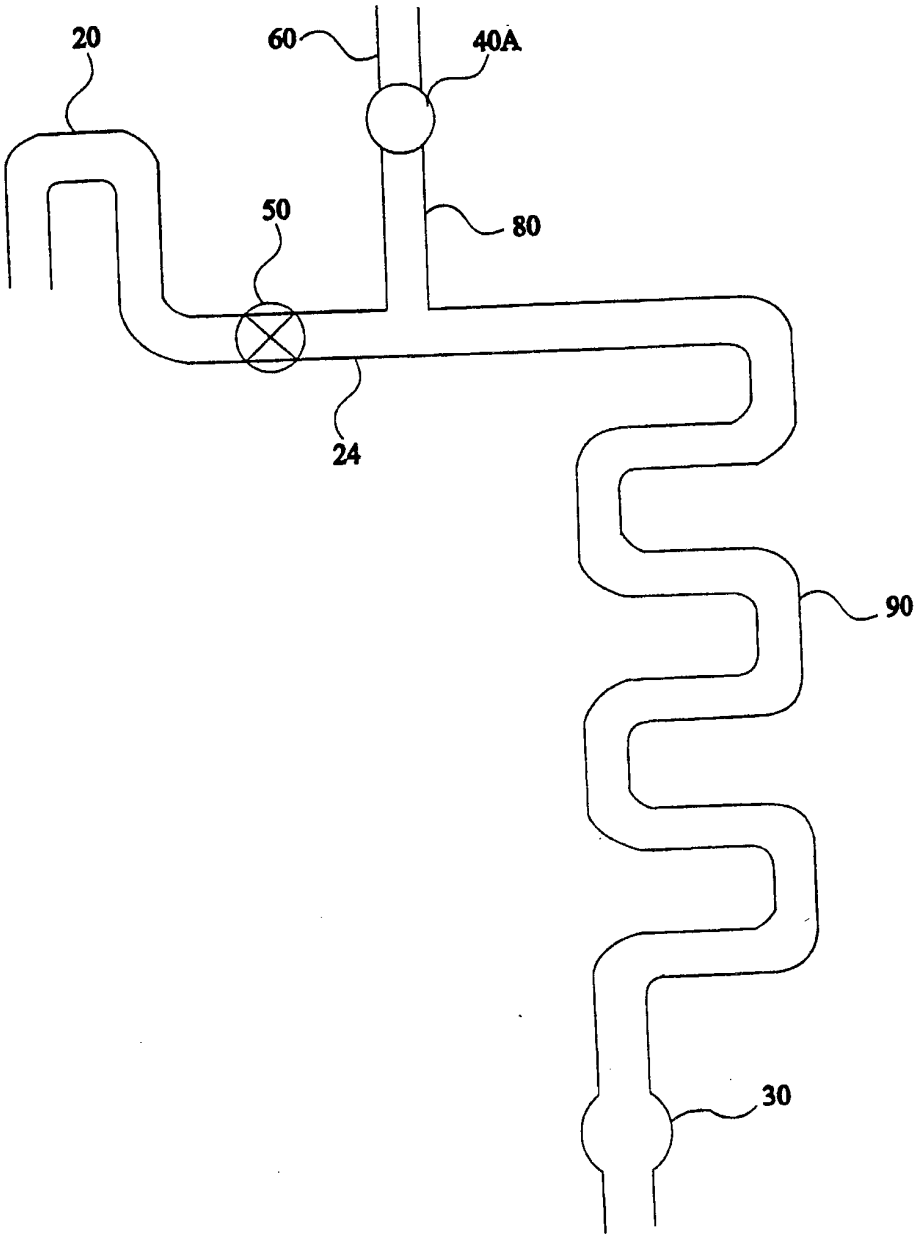


FIG. 9

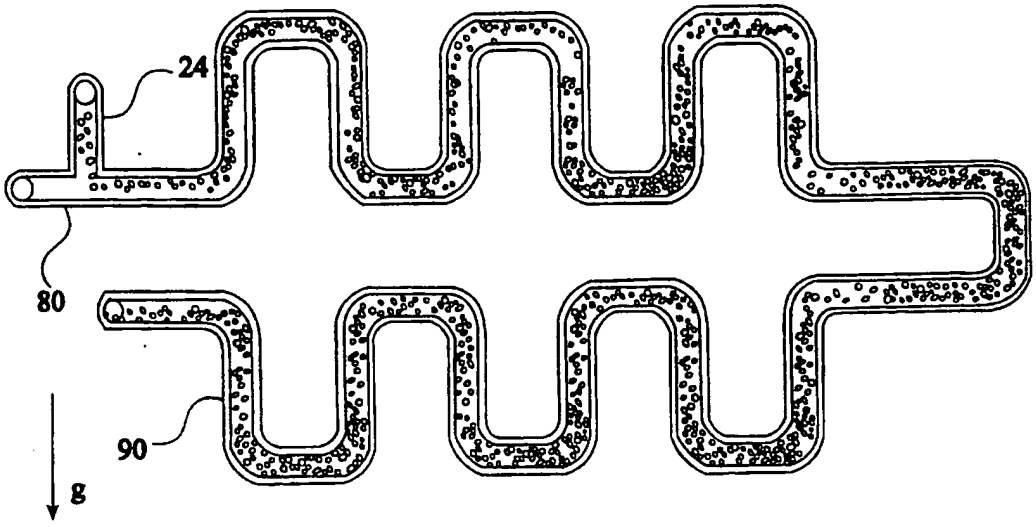


FIG. 10A

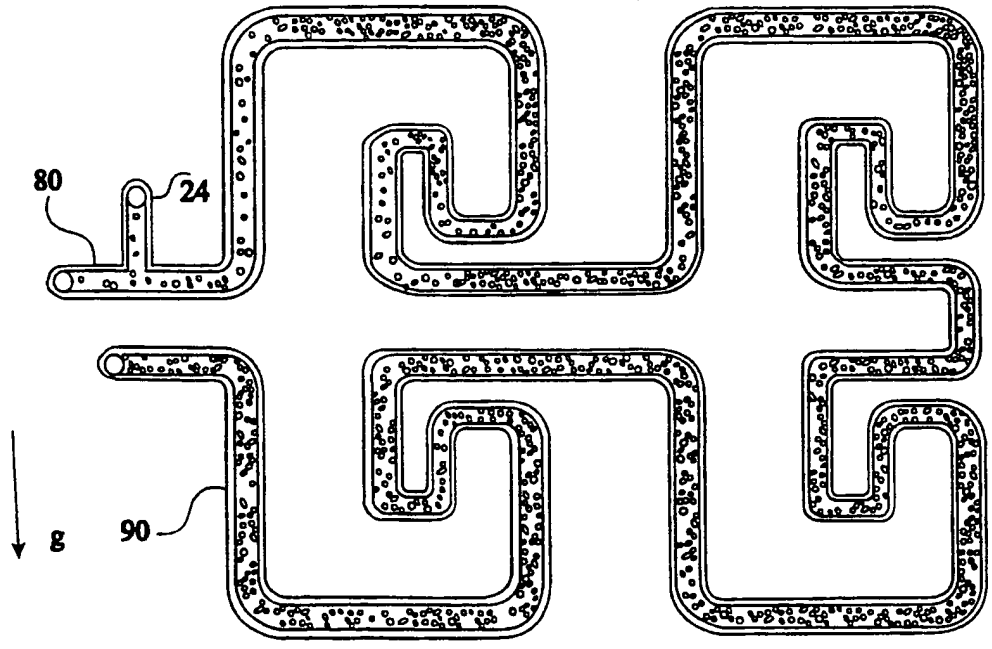


FIG. 10B

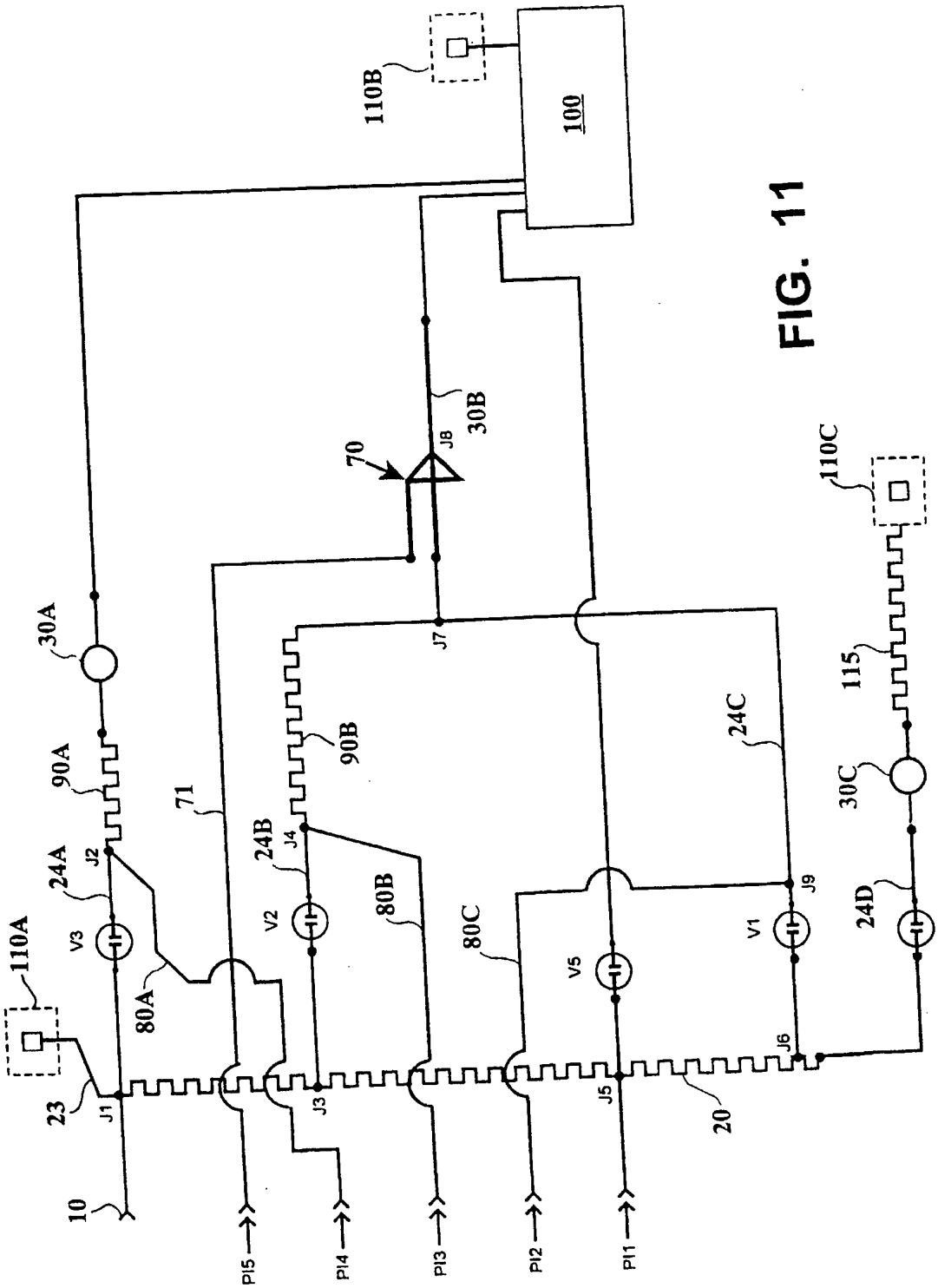

110B
100
30B
J8
70
110C
30A
J7
115
90A
90B
24C
30C
71
24B
J4
J2
24A
J9
V3
V2
80B
80C
V1
24D
110A
V5
80A
J6
23
J1
J3
J5
20
10
PI5
PI4
PI3
PI2
PI1

FIG. 11

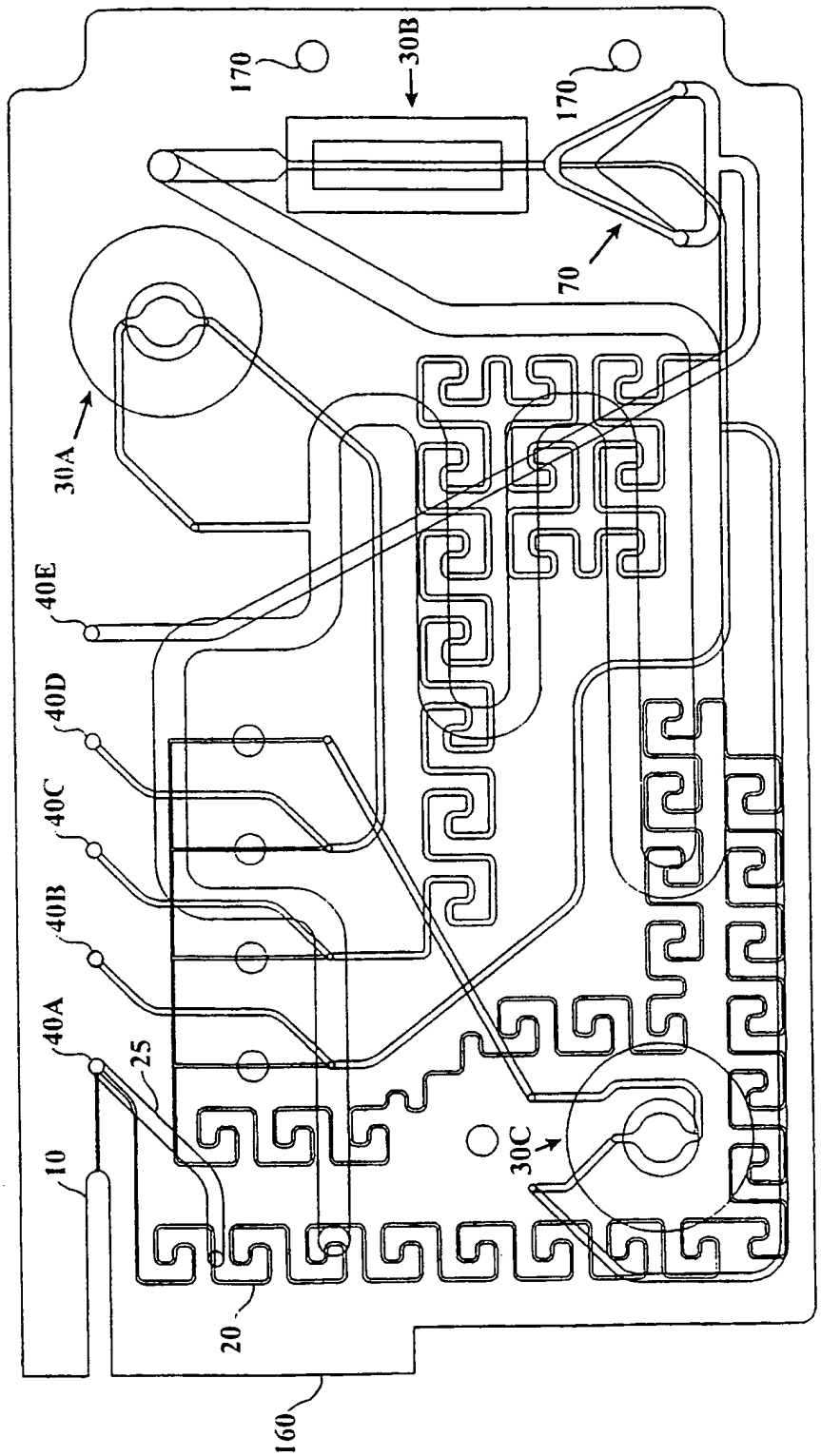


FIG. 12

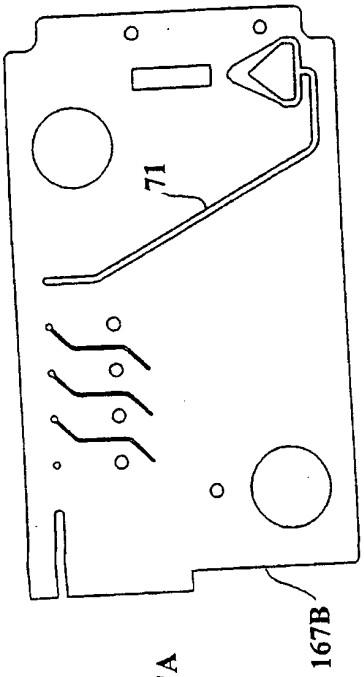


FIG. 13B

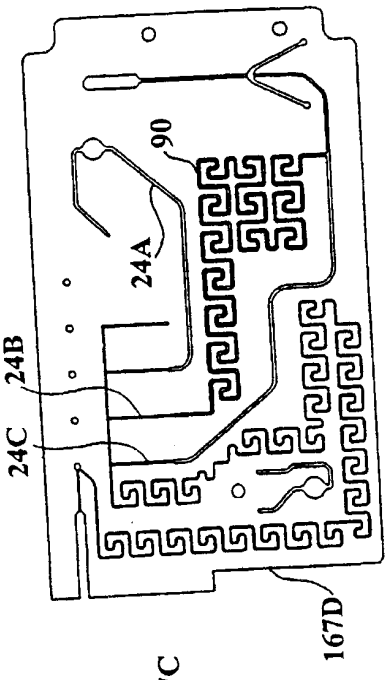


FIG. 13D

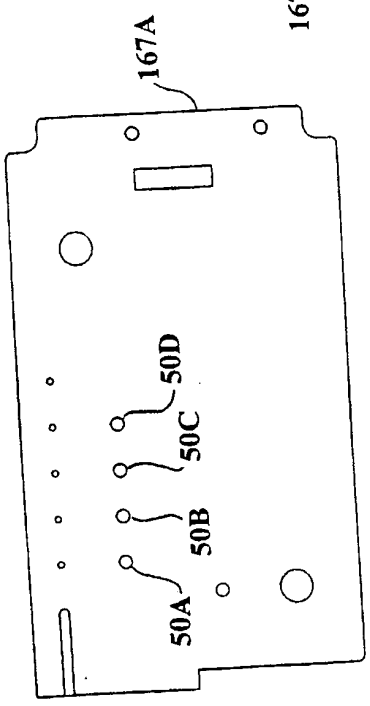


FIG. 13A

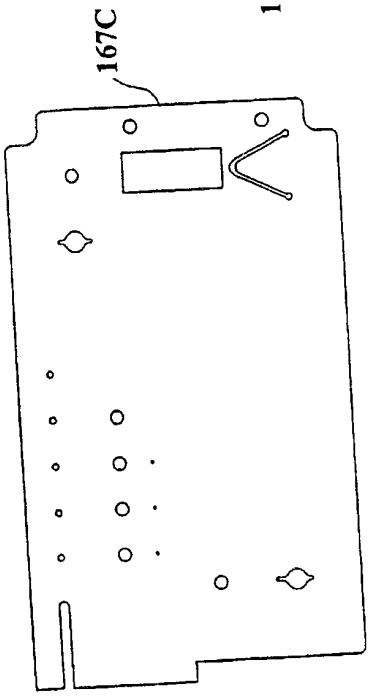


FIG. 13C

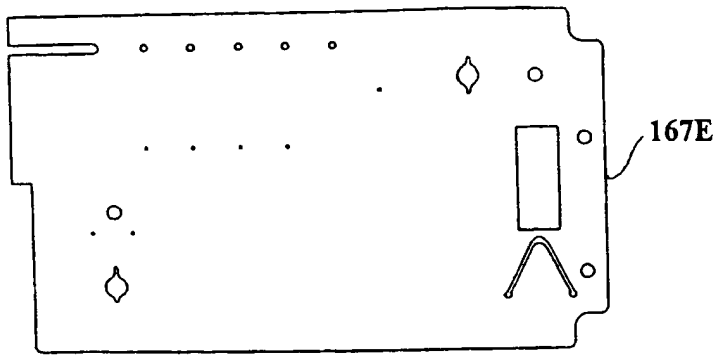


FIG. 13E

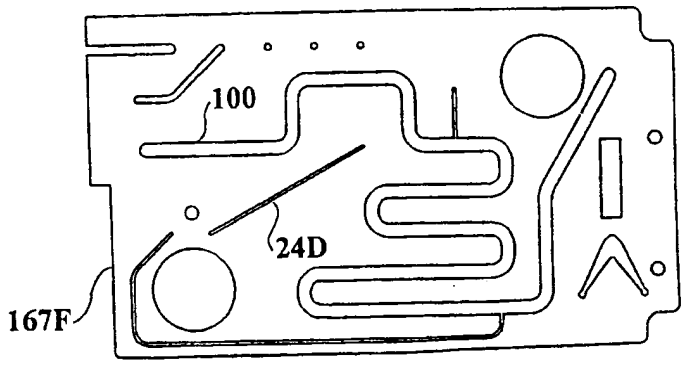


FIG. 13F

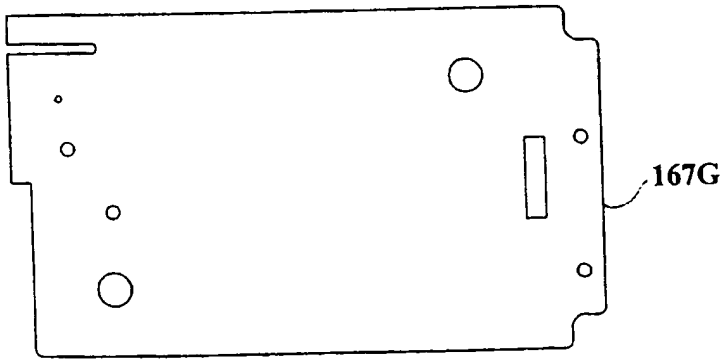


FIG. 13G

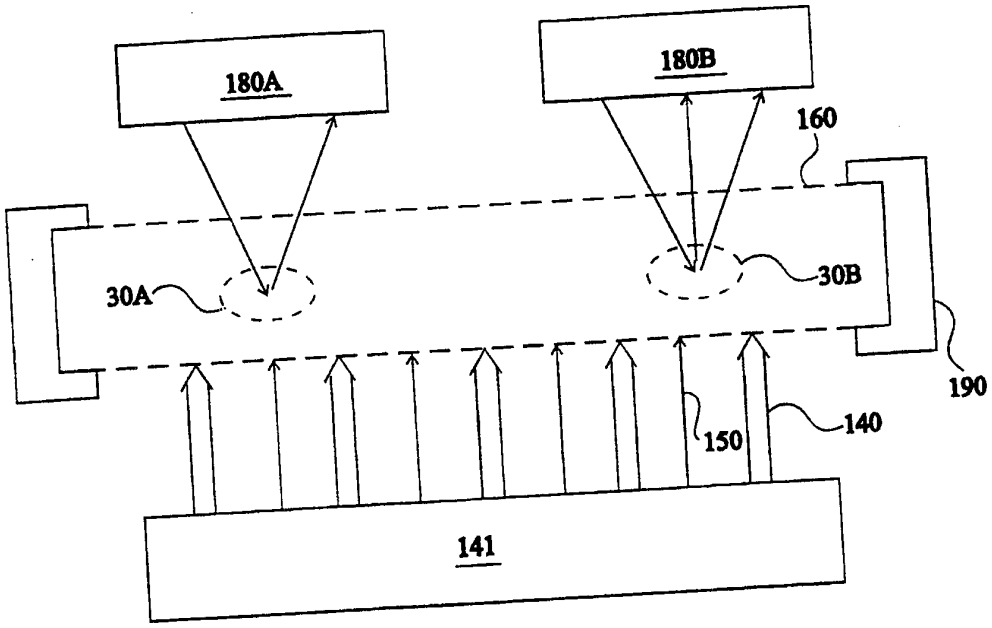


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09322

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/483, G01 21/01
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/58, 68.1, 81, 100, 82.05, 82.08, 104; 436/43, 164, 165, 172, 174, 179, 180

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,744,366 A (KRICKA et al) 28 April 1998, see whole document.	1-61,80-87,98-122
Y	US 5,500,187 A (DEOMS et al) 19 March 1996, col.10, lines 8-37 and col. 11, lines 1-21	88-97
Y	US 5,147,607 A (MOCHIDA) 15 September 1992, see whole document	1-61, 80-87,98-122
Y	US 5,726,751 A (ALTENDORF et al) 10 March 1998, col. 1-3	1-87 and 98-122
Y,P	US 5,755,942 A (ZANAUCCHI et al) 26 May 1998, see whole document.	72-79

[X] Further documents are listed in the continuation of Box C. [] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"B" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 SEPTEMBER 1999	19 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Patricia Lex Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09322

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,E	US 5,919,711 A (BOYD et al) 06 July 1999, see whole document.	1-122
A,P	US 5,863,502 A (SOUTHGATE et al) 26 January 1999, see whole document.	1-122

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09322

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09322

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/58, 68.1, 81, 100, 82.05, 82.08, 104; 436/43, 164, 165, 172, 174, 179, 180

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-39, drawn to a test device with a convoluted sample storage channel
Group II, claim(s) 40-61, drawn to a device with a flow cytometric measuring channel.
Group III, claim(s) 62-97, drawn to a method for measuring both the light absorbance and scatter of blood.
Group IV, claim(s)98-105, drawn to a device having a resuspension means.
Group V, claim(s) 106-118, drawn to a laminated fludic flow cartridge and method of manufactor.

The inventions listed as Groups I,II,III,IV and V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I has the special technical feature of a convoluted sample storage channel that is not in any of the other groups. Group II has the special technical feature of a flow cytometry measuring channel. Group III has the special technical feature of means for measuring the light abosorbance and scattering of blood. Group IV has the special technical feature of a resuspension pump. Group V has the special technical features of a laminated fluidic flow cartridge and method of making.